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SECOND CONFERENCE ON SULFONAMIDES

BY

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Consulting Editor

PERRIN H. LONG

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* This series of papers is the result of the *Second Conference on Sulfonamides* held by the Section of Biology of The New York Academy of Sciences, March 22, 1957. See *Sulfonamides*, Vol. 44, Art. 5, based on the previous conference held by the Academy, April 16 and 17, 1943.

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INTRODUCTION OF GERHARD DOMAGK

By Perrin H. Long
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The work of Gerhard Domagk, discoverer of the antibacterial activity of sulfonamide compounds, was first published in 1935. It marked the beginning of the epoch in which, by the use of either chemotherapeutic compounds or antibiotic agents, therapeutic control of the major infectious diseases produced by bacterial invaders and, in certain instances, by protozoa, has been achieved. Domagk's discovery and the subsequent work of scientists in other countries robbed streptococcal and meningococcal meningitis of their terrors, reduced the case fatality rate in pneumococcal pneumonia by two thirds, provided an entirely new approach to the treatment of urinary tract infections, and brought about prompt recovery in a host of other infectious processes. The fact that sulfonamides had proved their effectiveness spurred Howard Florey and his group to the study of other sources of antibacterial agents, which led, in turn, to the discovery of penicillin. Truly, Domagk's work opened a new chapter in man's long fight against disease and death.

In recent years, however, as one "miracle" antibiotic has succeeded another, sulfonamides, while still widely used, no longer are given the attention they once received. For this reason, and because of the development of two new sulfonamide compounds, it was considered desirable and reasonable to scientists and clinicians to hold a conference in order to re-evaluate the use of sulfonamides in present-day therapeutics. Domagk's contribution to this monograph, based on that conference, is particularly welcome.

TWENTY-FIVE YEARS OF SULFONAMIDE THERAPY

By Gerhard Domagk
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In December 1932 the unequivocally curative action of Prontosil had been observed in many experimental streptococcal infections. This was reported to the chemists Mietzsch and Klarer,[1] who had produced the compound for chemotherapeutic applications, and a large-scale joint effort was undertaken to investigate this field. Shortly afterward an announcement was made at the German Meeting of Scientists and Physicians in Königsberg that Prontosil and related sulfonamide compounds had proved effective also in experimental pneumococcal and staphylococcal infections. On the 15th of February 1935 the first announcement was made in the *Deutsche Medizinische Wochenschrift* regarding the experimental and clinical effects of this compound, the first sulfonamide recognized to be of practical value.

Shortly after the publication of our results, intensive research in this field was undertaken in othe countries. In London, England, exhaustive objective tests were carried out by the Medical Research Council to check the curative results described by Klee and his collaborators,[2] with particular regard to the effect in puerperal sepsis and other puerperal infections. The unequivocal value of the new remedy for this important indication was definitely established by Colebrook and his fellow workers.[3] Since that time the death rate from puerperal sepsis and septic abortions has continually decreased, both in Germany and in England, so that the unforeseen and so often fatal sickness of young mothers, to the control of which Semmelweis[4] was the first to devote and sacrifice himself, has now almost completely disappeared. The introduction of antibiotics, particularly penicillin, combined with sulfonamides or streptomycin, insured still greater safety and reduced the time required for successful treatment.

With the introduction of *p*-(*p'*-aminobenzenesulfonamido)benzenesulfonedimethylamide came the first colorless sulfonamide for the treatment of gonorrhea and meningitis epidemica; this preparation, however, was largely replaced by other sulfonamides, in which a hydrogen atom in the sulfonamide group was replaced by a heterocyclic ring, such as the pyrimidine or thiazole ring. Before the appearance of sulfonamide-resistant strains and their propagation during the war and in the postwar years, important dermatologists believed that gonorrhea could be eradicated by an oral treatment with sulfonamides alone. As a result of sulfonamide and penicillin treatment, gonorrhea has now become a rare, if not yet entirely eradicated, disease.

The introduction of sulfonamides for the treatment of meningitis epidemica was even more satisfactory, since it was instrumental in saving lives. By oral administration alone, pediatricians were successful in developing this treatment to the extent that death due to meningitis epidemica became a rarity, whereas after the introduction of the serum treatment, mortality still exceeded 50 per cent. Only a few years after the introduction of the sul-

fonamide treatment the number of children whose lives had been saved in England alone was estimated at ten thousand.

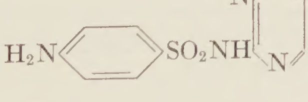
Hardly less impressive was the effect of sulfonamide treatment on the mortality from acute pneumococcal pneumonia. This disease, which twenty to thirty years ago was still one of the most frequent causes of death even among young people, is now under complete control thanks to chemotherapy. Many students are therefore no longer acquainted with the typical stages of lobar pneumonia except from descriptions in textbooks or specimens from older anatomical collections. Before the introduction of the serum treatment, aside from such methods as stimulation of the circulation with camphor, virtually only physical expedients were available; and even the serum treatment of pneumonia presented so many difficulties that it could hardly be applied on a wider scale.

Bacterial dysentery, the most dreaded pestilence of war, lost its terrors after the introduction of sulfonamide treatment, not only for the individual, but also as an epidemic.[5]

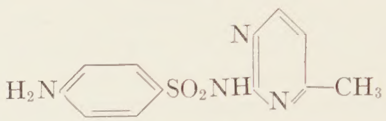
We could mention many other diseases, the characteristics of which have changed since the introduction of sulfonamides. For the first time these drugs have enabled us to attack these diseases with causal treatment.

With the large number of sulfonamides and antibiotics now available as effective remedies, they are unfortunately sometimes subject to abuse, since they are even applied in simple and absolutely uncomplicated infections such as occur, for instance, in pharyngitis. This is going too far, and it may lead, in the long run, to an incompatibility that will render this method of treatment impossible in a serious case where application of the chemotherapeutic or antibiotic agent is indicated. On the whole, after twenty-five years of application, the sulfonamides have given rise to relatively few serious secondary effects, even when compared with the new antibiotics in use for such a short time. Moreover, the secondary effects caused by sulfonamides could be considerably reduced, or even completely eliminated, if the exaggerated doses now administered could again be restored to smaller yet fully effective doses.

Unfortunately, it is often emphasized that one remedy or another is tolerated in doses up to 10 to 12 gm. without consideration of its relative efficiency. The essential point is not the absolutely tolerable dose of a less effective remedy, but the ratio of the effective dose to the tolerable dose. In this connection there is no doubt that some of the pyrimidine-containing sulfonamides lead the field; two of them are particularly outstanding when compared with many others also recommended for medical use. At present, the most effective sulfonamides are 2-(*p*-aminobenzenesulfonamido)pyrimidine,



known also as debenal, pyrimal, and sulfadiazine, and also 2-(*p*-aminobenzenesulfonamido)4-methylpyrimidine,



known also as methyldebenal or sulfamerazine.

The best test, and indeed the only test, for accurately assessing the value of a sulfonamide is to carry out experiments on infected animals. It was by this means that my associates and I discovered and further developed the curative properties of the sulfonamides. The wide differences in the sulfonamide types still being used can be seen in the results presented in TABLE 1, which illustrates the great superiority of sulfadiazine or debenal over other sulfonamides also containing pyrimidine.

This brief example, which is fully confirmed by a large number of experimental results in international literature (in the United States particularly by Lehr[6] and his collaborators, and also by Sophian, Piper, and Schneller[7]), shows that, of the sulfonamides of the pyrimidine class, the most effective by far are sulfadiazine (debenal) and sulfamerazine (debenal M). Using the relatively low dosage of 3 to 5 gm. of these sulfonamides, at least the same clinical results are achieved as with twice the dose of other sulfonamides, but with the difference that no secondary effects need be anticipated, due, for example, to overdosing. Unfortunately, the most effective sulfonamides have been somewhat discredited by improper overdosing and have been replaced, quite unjustifiably, by other much less valuable sulfonamides. We could get along quite well with the two most effective sulfonamides. Even the presently popular triple sulfonamides, in my opinion, would be quite unnecessary if the most effective sulfonamides were used in the minimum doses required to insure an optimum effect. If, however, larger doses are to be administered with the assurance

TABLE 1

	Number of animals	Number alive 24 hr. after infection	Number alive 48 hr. after infection	Number alive 1 wk. after infection		
Controls	20	0	0	0		
*Sulfadimetine**						
1 % S.C.	10	10	2	0	0	3
1 % *per os*	10	7	1	0		
5 % S.C.	10	6	3	1	3	
5 % *per os*	10	9	6	2		
Debenal						
1 % S.C.	10	9	8	3	3	13
1 % *per os*	10	9	3	0		
5 % S.C.	10	10	9	6	10	
5 % *per os*	10	8	8	4		

Mice infected I.P. with β-hemolytic streptococci (4/1/55). One administration 1 hr. after infection. Dose 1 or 5 per cent for each pair of animals: 0.2, 0.5, 0.8, 1.0 cc. administered S.C. or *per os*.
*Sulfadimetine = 6-sulfa-2,4-dimethylpyrimidine.

that precipitation will not take place in the urinary tubules, then, in these cases at least, the most effective sulfonamides should be used in preference to preparations the application of which should now be regarded as superseded. In exceptional cases, where failure is experienced with the leading preparations, they could then be combined with the orally effective penicillin, thus avoiding the risk associated with penicillin injections. The number of deaths attributed in part to penicillin was stated to be six hundred in one year alone (Halpern[8]). Given correct application of so valuable a remedy, these deaths most surely could be avoided—without impairing the curative effect—by combining the simple, harmless, orally administered penicillin with sulfonamides.

Since injections are always less popular with patients than with physicians, due to their disagreeable effects when compared with oral administration, the latter method, if possible, should always be preferred. Moreover, the practitioner who is unable to sterilize his syringes in a drying cabinet at 180° C. runs a not inconsiderable risk of transmitting an infection, particularly in the case of virus infections. Aside from irrelevant considerations, a remedy should be regarded, in most cases, as superior only if it can be administered orally and is also both tolerable and effective. In staphylococcal infections, even those with penicillin-resistant strains, our experience has shown that the combined administration of debenal plus chloromycetin (leukomycin) is particularly effective.

Now, more than twenty years after the introduction of the first sulfonamide to the medical profession, we have available several sulfonamides and antibiotics that, in small doses, are highly effective clinically and that enable us largely to control the majority of clinically important infectious diseases. Our future task is therefore to investigate further, etiologically, those infectious diseases still incurable or little amenable to treatment, so that a basis may be established for curing them by chemotherapeutic intervention.

The following reasons for the present increasing importance of sulfonamides are given by Lehr[6] of the New York Medical College, New York, N. Y.: (1) improvement in the effect obtained due to better preparations; (2) increasing knowledge regarding certain great disadvantages of the antibiotics, particularly in gastrointestinal disorders, staphylococcal diarrhea, moniliasis, and colitis pseudomembranosis, which can be relieved by the broad spectrum-antibiotics; and (3) high antibacterial effect with such advantages as economy and exact determinability in the body fluids.

A. M. Walter gives the following reasons for the greatly renewed interest shown in sulfonamides during 1956:

(1) The "more compatible" antibiotics have turned out to be remedies that are certainly not without danger. The frequently fatal course of enteritis ulceromembranacea and severe allergic damage may be considered warning signs.

(2) Further development of the sulfonamides has created more effective and more pharmacologically suitable compounds that are practically free from risk.

(3) The wide application of oral administration in the case of sulfonamides is more agreeable to the patients.

(4) The price of sulfonamides has been considerably reduced.

Whether a treatment should be given with sulfonamides or antibiotics must be decided in each individual case. In any case, it is advisable first to administer, in reasonable—not exaggerated—doses, well-tried sulfonamides that, with their slight side effects, have been used for decades; and then, in the event of failure, to resort to the more effective remedies that involve a greater element of risk.

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CURRENT USAGE OF SULFONAMIDES IN THE TREATMENT OF INFECTIONS IN ADULT PATIENTS

By Perrin H. Long

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In a consideration of the usage of sulfonamides in the treatment of infections in adult patients, it is necessary to determine those infections and other conditions in which, at present, sulfonamides are favored over the antibiotics as therapeutic agents.

This is a difficult problem, because there can be wide areas of disagreement between individuals interested in the treatment of infectious processes; and I suspect that many of my colleagues will disagree with my opinions. For the record, I shall say that sulfonamides as the sole therapy are indicated in the treatment of many instances of uncomplicated urinary tract infection, in bacillary dysentery, in meningococcal infections, in trachoma, and as prophylactic agents in the preparation of the bowel for surgical procedures. Prescription of a sulfonamide compound has also brought relief to many victims afflicted with dermatitis herpetiformis. Sulfadiazine is still the antibacterial agent of choice for the prophylaxis of meningococcal meningitis.

There is also what I shall call a secondary use for sulfonamides, namely in the treatment of infections, the causative organisms of which have proved to be resistant to the therapeutic effects of antibiotics. In this area it is again in the field of urinary tract infections that one most frequently encounters infecting organisms that are resistant to antibiotic therapy. Also, thought should be given to the use of certain of the sulfonamides in the treatment of staphylococcal infections that are resistant to therapy with currently available antibiotics. As far as staphylococci are concerned, most of the sulfonamides that are absorbed systemically are bacteriostatic. Certainly all remember, in the days before the discovery of antibiotics, patients suffering from severe staphylococcal infections in whom the balance was tipped favorably following the administion of sulfonamides.

Finally, one must consider the combined use of sulfonamides and antibiotics. Certainly, it is known from studies on the mode of action of sulfonamides and at least some of the antibiotics that these antibacterial compounds exercise their detrimental effect on different systems in the bacterial cell. Hence, when one uses a sulfonamide concurrently with an antibiotic, one should achieve minimally an additive therapeutic effect. However, when an antibiotic and sulfonamide are used, fixed combinations of the two cannot be recommended. Rather, the amount of each therapeutic agent that should be maximally effective should be calculated and then administered separately.

The question of whether to use a single sulfonamide or a mixture of two or more sulfonamides for the treatment of an infectious process is a matter for

decision by the physician, although, certainly in meningococcal infections, the bulk of evidence indicates that sulfadiazine is the sulfonamide of choice.

The dosages to be employed when sulfonamides are being used must be graded to give a concentration of free sulfonamide of from 10 to 15 mg. per cent in severe infections, and from 5 to 10 mg. per cent in moderately severe infections.

CURRENT USES OF SULFONAMIDES IN PEDIATRIC PRACTICE

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The introduction of penicillin and streptomycin greatly reduced the use of sulfonamides in pediatrics. This trend was accentuated by the discovery of chloramphenicol, the tetracycline drugs, and other antibiotics that are effective when taken by mouth, and by the development of effective oral preparations of penicillin. Nevertheless, the sulfonamide drugs still have a definite place in the therapy of infectious diseases of infancy and childhood. In most instances their role is that of an auxiliary to the generally more effective antibiotics. For treatment of infections caused by meningococci, however, they are the most effective agent available. The use of sulfonamides together with penicillin improves the chance for recovery from pneumococcic meningitis, and the compounds are probably of some value when used with chloramphenicol in the treatment of *Hemophilus influenzae* meningitis.

The sulfonamides are of definite value in the treatment of infections of the urinary tract. Many pediatricians begin the treatment of pyelonephritis with sulfonamides and change to antibiotics only if the clinical course is not satisfactory or if bacteriological studies indicate a need for such change.

Sulfonamides are still employed by many physicians for treatment of gastrointestinal infections due to *Shigella* organisms, as well as for infections of less clearly established etiology. Sulfasuxidine and sulfathalidine are most commonly used for this purpose, but many pediatricians employ sulfadiazine and Gantrisin for these infections. Sulfonamides should not be used for treatment of *Salmonella* infections, since chloramphenicol is much superior for this purpose, although it is not as effective as the literature indicates. Similarly, neomycin (given by mouth) is superior to sulfonamides for infantile diarrhea caused by enteropathogenic types of *Escherichia coli*.

Perhaps the most difficult problem regarding the sulfonamides concerns their use in the treatment of infections of the upper respiratory tract. In practice, the pediatrician usually must begin treatment without definite knowledge of the etiological agent. Often he will be forced to treat an infection caused by a virus; in this situation neither sulfonamide nor antibiotic will have a curative effect, although secondary bacterial infection may be prevented. Sometimes, however, the pediatrician may have a fairly good idea that he is dealing with a group A β-hemolytic streptococcus (a sibling may have scarlet fever, for example). Sulfonamides alone should not be used in such cases, nor in others in which the probability is great that a hemolytic streptococcus is the causative agent. Instead, penicillin should be used, because of its greater effectiveness in preventing the occurrence of rheumatic fever and nephritis. We believe that sulfonamide should not be given if a bacterial infection of any kind has extended from the pharynx and tonsils to the middle ear, sinuses, larynx, or trachea, but rather that an antibiotic should be used. This recommendation

is based upon two considerations. First, sulfonamides are relatively ineffective when pus is present, a condition that is liable to occur with middle-ear and sinus infections. Second, infections of the organs mentioned are potentially dangerous, and the most effective agent available should be chosen. Usually this will mean the choice of an antibiotic rather than a sulfonamide.

Some pediatricians regularly employ an antibiotic together with a sulfonamide for treatment of upper respiratory tract infections. Their reason is that such a combination broadens the antibacterial coverage, that over-all results of therapy are better, and that the cost to the patient in money and in risk of toxic effects is very small. This is possibly the case in many instances, but we believe that this practice is not as satisfactory as the use of a broad spectrum antibiotic (as tetracycline) alone.

Sulfonamides may be used as prophylactic agents to prevent secondary infection in certain viral infections of infants and very young children. Measles is an example of such an infection. It may be argued with considerable validity that the low cost of a sulfonamide makes it the drug of choice in this situation. It is the drug of choice in prophylaxis of children exposed to meningococci. Sulfonamides may also be used for prophylaxis against β-hemolytic streptococcal infections for children who have suffered an attack of rheumatic fever. They may not be quite as effective for this purpose as certain preparations of penicillin suitable for oral use, but a decision on this point cannot yet be made with certainty. The low cost and ease of administration of sulfonamides must be considered here, and it is clear that, by and large, the sulfonamides have proved to be effective prophylactic agents against group A streptococci.

In conclusion, sulfonamides still have a role in the treatment of infectious diseases of infancy and childhood. In a limited number of infections a sulfonamide is the drug of choice, but in most instances it is an auxiliary agent to be used in conjunction with antibiotics. In certain infectious diseases its value even as an auxiliary agent is doubtful.

THE CLINICAL USE OF SULFISOMIDINE IN URINARY TRACT INFECTIONS

By Alexander M. Rutenburg

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In 1952 my associates and I[1] reported on the therapeutic efficacy of sulfisomidine (Elkosin) in a group of 40 unselected patients with acute and chronic urinary tract infections due to the commonly encountered variety of Gram-negative and Gram-positive bacteria.

This report deals with a brief review of our original study and the therapeutic effect of sulfisomidine in 55 additional patients with urinary tract infections due mostly to gram-negative bacteria.

In 1951–1952 the comparative *in vitro* sensitivity of 88 strains of various bacterial species to sulfisomidine, sulfadiazine, sulfamethazine, and sulfacetimide was determined by a standardized serial tube dilution method.[2, 3] With this method the bactericidal titer was taken to be the lowest concentration required to prevent macroscopically visible growth at 37° C. in 24-hr. cultures that, on subculture for 48 hr., did not reveal any growth. A strain was considered sensitive, that is to say, clinically eradicable by the usual therapeutic doses of sulfonamides, if the bactericidal titer *in vitro* by this method was 250 mg. per cent or less, and was considered resistant if more than 250 mg. per cent.

Results of these tests indicated that sulfisomidine and sulfamethazine were generally effective against most strains of Gram-positive and Gram-negative microorganisms and were clearly superior to sulfadiazine and sulfacetimide (TABLES 1 and 2). TABLE 1 demonstrates not only the familiar fact that different strains of a given bacterial species vary widely in their sensitivity to the same and to the various sulfonamides, but that sulfisomidine is superior to other sulfonamides. TABLE 2 provides detailed data on the range of sensitivity of various strains within a species.

In the original group of 40 patients with urinary infections, 20 of the infections were due to a single organism and 20 were due to more than one. *Escherichia coli* was isolated in 22 patients, *Pseudomonas aeruginosa* in 15, *Proteus vulgaris* in 11, staphylococci in 3, streptococci in 2, and diphtheroids in 1.

All patients had pyuria, positive urine cultures, and the usual signs and symptoms. Twenty-four had calculi, stricture, prostatic obstruction, or were on constant drainage. Obstructive lesions, if present, were treated, and repeated catheterizations or constant drainage were discontinued during sulfisomidine therapy.

The results were evaluated on a clinical and bacteriological basis after a follow-up period varying from 12 to 271 days. If the urine became sterile and all symptoms disappeared, the final response was classified as cured. If those patients with polyvalent infections improved, and the count of a bacterial strain was markedly reduced, even though the urine was not sterilized, the response was classified as good. If there was no bacteriological improvement,

Table 1

Comparative *in Vitro* Sensitivity of Bacteria to Sulfisomidine, Sulfamethazine, Sulfadiazine, and Sulfacetimide

Species	Total number of strains	Sensitivity to sulfisomidine as compared to other sulfonamides			Resistant to all 4 sulfonamides
		More sensitive	Equally sensitive	Less sensitive	
		Number of strains	Number of strains	Number of strains	Number of strains
E. coli	11	4	3	1	3
A. aerogenes	8	4	0	1	3
Ps. aeruginosa	20	2	3	4	11
P. vulgaris	15	1	0	3	11
Staph. aureus	15	3	2	2	8
β-hemolytic streptococci	6	3	2	0	1
Salmonella	9	5	4	0	0
Shigella	4	0	4	0	0
Total	88	22	18	11	37

Table 2

Comparative *in Vitro* Sensitivity of Several Strains of a Given Species of Bacteria to Four Sulfonamides*

Strain	Bactericidal titer in mg.% of drug in nutrient broth				Number of strains within each type of sensitivity
	Sulfisomidine	Sulfamethazine	Sulfacetimide	Sulfadiazine	
E. coli I	50	100	250	1,000	4
E. coli II	50	50	500	1,000	3
E. coli III	500	250	500	1,000	1
E. coli IV	750	750	750	1,000	3
A. aerogenes I	250	500	500	1,000	4
A. aerogenes II	500	100	500	1,000	1
A. aerogenes III	750	750	750	750	3
Ps. aeruginosa I	250	1,000	1,000	1,000	2
Ps. aeruginosa II	250	250	500	1,000	3
Ps. aeruginosa III	750	250	750	1,000	4
Ps. aeruginosa IV	1,000	1,000	1,000	1,000	11
P. vulgaris I	250	1,000	1,000	1,000	1
P. vulgaris II	750	500	1,000	1,000	3
P. vulgaris III	750	750	1,000	1,000	11
Staph. aureus I	250	500	500	500	3
Staph. aureus II	250	250	500	1,000	2
Staph. aureus III	250	100	750	500	2
Staph. aureus IV	1,000	1,000	1,000	1,000	8
β-hemolytic *Streptococcus* I	100	250	250	500	3
β-hemolytic *Streptococcus* II	50	50	250	250	2
β-hemolytic *Streptococcus* III	750	750	750	1,000	1
Salmonella I	250	750	750	750	5
Salmonella II	500	500	750	1,000	4
Shigella I	100	100	250	500	4

* Size of inoculum, 2000 to 8000 bacteria.

or if the original flora disappeared but was replaced by other resistant strains, the response was classified as failure.

Twenty-four of these 40 patients were termed cured, and 3 others showed a good response with sulfisomidine. Of the 20 of the 27 patients responding to sulfisomidine, 8 had failed to benefit from prior therapy with penicillin, 8 from penicillin and chlortetracycline, 2 from chlortetracycline, 1 from penicillin and sulfamethazine, and 1 from chloramphenicol.

Sulfisomidine produced a satisfactory response in 13 of 20 patients with mixed infections, where sulfisoxazole, in our experience, proved of little or no value. We observed improvement in some patients with polyvalent as well as with monovalent infections. In the 20 patients with infections caused by a single organism, the urine was sterilized in all but 6.

Sulfisomidine was absorbed rapidly from the gastrointestinal tract. Following a single oral dose of 2 gm. of sulfisomidine, a maximum blood concentration of free sulfonamide (6 to 8 mg. per cent) was reached in 2 to 3 hr., and levels of 2 to 6 mg. per cent were maintained for 4 to 6 hrs. thereafter. On a dosage of 4 gm. daily, blood levels of 6 to 12 mg. per cent of free sulfonamide were easily maintained. Determinations of free and total sulfonamide in the blood and urine of patients receiving this drug demonstrated a low order of acetylation, that is, 80 to 95 per cent of the total sulfonamide were in the free form.

Despite the fact that no attempt was made to maintain an adequate daily fluid intake or to alkalinize the urine, we met with no untoward reactions or renal or hematopoietic toxicity.

Clinical Observations

More recently the results of sulfisomidine therapy were evaluated in 55 additional patients with urinary tract infections, caused by a single organism in 39 patients and by more than one in 16. *E. coli* was isolated in 28 patients, *Aerobacter aerogenes* in 13, *Ps. aeruginosa* in 11, *P. vulgaris* in 11, streptococci in 9, and staphylococci in 5.

Thirty-seven of the patients were ambulatory or were hospitalized because of a primary urinary tract infection, whereas the remaining 18 developed their urinary infection in the hospital following operations, repeated catheterization or instrumentation, or while on constant catheter drainage.

An initial oral dose of 2 gm. of sulfisomidine was followed by 4 gm. daily in divided doses. Alkali was not given and no attempt was made to maintain a minimum daily fluid intake. Most of the patients were treated for 5 days. If there was no improvement in 10 days, or if the responsible bacteria were found to be resistant *in vitro*, the drug was considered ineffective and was discontinued.

Toxic reactions were not observed. Renal damage, crystalluria, or hematuria did not occur. Gastrointestinal or allergic reactions were not noted. Anemia, leukopenia, or agranulocytosis did not develop.

Results

The results were evaluated on the basis of the criteria described previously. Thirty-five of the 55 patients showed a satisfactory response to therapy: 31

TABLE 3
RESULTS OF SULFISOMIDINE THERAPY IN 55 PATIENTS WITH URINARY INFECTIONS

	Number of patients	Result		
		Cured	Good	Failure
Monovalent infections	39	26	—	13
Polyvalent infections	16	5	4	7

were cured, 4 showed a good response (TABLE 3). Twenty patients were classified as failures.

Of the 39 monovalent infections, there were 13 in which the urine could not be sterilized. Four were due to *E. coli*, 3 to *Ps. aeruginosa*, and 2 each to *A. aerogenes*, *P. vulgaris*, and staphylococci.

Of the 16 mixed infections, the urine was sterilized in 5. Four others showed clinical and partial bacteriological improvement. Seven did not respond to sulfisomidine.

Of the 77 bacterial strains found in the urines of 55 patients, sulfisomidine eliminated 44 (19 of 28 strains of *E. coli*, 7 of 9 strains of streptococci, 7 of 13 strains of *A. aerogenes*, 5 of 11 strains of *P. vulgaris*, 4 of 11 strains of *Ps. aeruginosa*, and 2 of 5 strains of staphylococci) In 4 patients the original flora was replaced during therapy by resistant bacterial strains.

A comparison of the results in the 37 ambulatory patients or those who were hospitalized with primary urinary tract infections with those in the 18 patients who acquired urinary infection in the hospital revealed that 31 of the former (84 per cent) and only 4 of the latter group (22 per cent) showed a satisfactory response to sulfisomidine therapy.

In order to evaluate this increase in bacterial resistance, a comparison was made of the results of the *in vitro* sensitivity of bacterial strains isolated from infected urines of hospitalized patients in 1947, 1951, and 1955.[4] TABLE 4

TABLE 4
IN VITRO SENSITIVITY OF MICROORGANISMS RECOVERED FROM INFECTED URINES IN 1947–1948, 1951, AND 1955 TO SULFADIAZINE, SULFAMETHAZINE, SULFISOMIDINE, GANTRISIN, AND THIOSULFIL

Species	1947–1948		1951		1955	
	Number of strains tested	Percentage of strains sensitive to one or more sulfonamides*	Number of strains tested	Percentage of strains sensitive to one or more sulfonamides*	Number of strains tested	Percentage of strains sensitive to one or more sulfonamides*
Staph. aureus	10	80	15	47	11	0
Streptococci (α and β)	10	100	6	84	9	22
E. coli	30	100	11	74	19	0
A. aerogenes	10	100	8	63	12	0
Ps. aeruginosa	30	73	20	45	12	0
Proteus	30	73	15	27	10	20
Total	120	85	75	51	73	5

* Macroscopically visible bacterial growth was completely inhibited by a sulfonamide concentration of 100 mg. per cent or less.

shows that, in 1947–1948, 85 per cent of the total strains isolated were sensitive to 1 or more sulfonamides. In 1951–1952 about half of the 75 strains isolated were sensitive, and in 1955 only 4 strains of streptococci and *P. vulgaris* were responsive, whereas 69 of 73 strains of various bacterial species cultured from the urines of hospitalized patients with urinary infections were resistant to all sulfonamides tested. The results of these *in vitro* sensitivity tests correlated closely with the poor clinical response to therapy as reflected in the failure of patients with urinary tract infections, from which these resistant bacteria were isolated, to respond to sulfonamide therapy.

Bacterial strains from the urines of ambulatory patients and patients with primary urinary tract infections were less resistant *in vitro* to the sulfonamides than were bacteria encountered in hospitalized patients with secondarily acquired urinary infections.

Discussion

Bacteria that were sensitive *in vitro* to sulfisomidine were always eliminated from the urine. Bacteria that were resistant *in vitro* were also resistant *in vivo*. There was, therefore, a correlation between the clinical effect and the *in vitro* sensitivity of the infecting bacteria to the drug. Thus, *in vitro* sensitivity could be used before treatment as a reliable test for the clinical effectiveness of the antibacterial activity of sulfisomidine.

A study of *in vitro* sensitivity of bacteria isolated from infected urines of hospitalized patients to sulfonamides and antibiotics[4] reflected a progressive rise from 1947 to 1956 in the incidence of resistant strains.

The increased resistance of bacteria closely paralleled the intensive and often unnecessary use of antibiotics. The appearance of strains resistant to the tetracyclines and sulfonamides reflected their extensive use. No such increase in resistance to the less commonly used antibiotics such as streptomycin, polymyxin, and chloromycetin was observed. Indeed, whereas in 1947 most strains of Gram-negative bacilli isolated from infected urines were resistant to streptomycin, there was a marked decrease in the incidence of streptomycin-resistant strains encountered in 1955. This increase in sensitivity paralleled the curtailed use of streptomycin on the surgical wards of the Beth Israel Hospital from 1949 to 1955.

Development of resistant strains and clinical therapeutic failure can also be attributed in part, to injudicious chemotherapy of infection in unfavorable cases, that is, in patients with obstructive uropathy or indwelling foreign bodies, or in those subjected to repeated catheterization or instrumentation. The prophylactic use of antibiotics did not alter the incidence of infection in such patients, but did yield development of resistant bacterial strains. Generally, it was not possible to sterilize the urine in patients with indwelling catheters. At best, the original bacterial strain, usually *E. coli* or enterococci, was replaced by resistant staphylococci, *Pseudomonas* or *Proteus* strains. In such instances there was a conversion of initially susceptible strains to those very difficult to control. Combinations of two or more drugs achieved nothing more than development of strains resistant to each agent, without a substantial reduction in the incidence or severity of infection.

Summary

A study of comparative *in vitro* sensitivity of a variety of Gram-positive and Gram-negative bacteria commonly encountered in urinary infections indicated that sulfisomidine (Elkosin) and sulfamethazine had greater antibacterial effect *in vitro* than other sulfonamides.

Clinical trial in 95 unselected patients with acute and chronic urinary infections yielded a satisfactory response in 62. There was a notable lack of toxicity and a low order of acetylation. With no attempt made to maintain an adequate daily fluid intake or alkalinization of the urine, no renal or hematopoietic toxicity occurred. Bacteria sensitive to sulfisomidine *in vitro* were always eliminated from the urine. Bacteria that resisted the drug in patients, or that appeared in the urine during therapy, were also resistant to the drug *in vitro*.

Sulfisomidine was more effective in ambulatory patients and in patients with primary urinary tract infections than in hospitalized patients with secondarily acquired urinary infections. In the latter group the responsible bacteria were generally resistant *in vitro* to sulfisomidine, as well as to other sulfonamides and antibiotics. This reflected the general rise in the incidence of drug-resistant bacteria encountered in hospitalized patients.

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CURRENT USE OF SULFONAMIDES IN UROLOGY

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Abundant experience has accumulated since Gerhard Domagk first made available a sulfonamide that would affect pathogenic bacteria in man. Before this time our interest had centered in the methenamines and, later, in the mandelic acid made popular by Rosenheim. These were strictly of interest to the urologist, since they both affected infections in the urinary tract only, dependent as they were on the change to formaldehyde in the urine by the methenamines, and the high acidification of the urine by mandelic acid.

The sulfonamides introduced a new, broader concept in that the antibacterial action took place, not only in the urine, but anywhere the blood carried it in sufficient concentration.

The prompt control of infection by the sulfonamides caused investigators to proclaim them as miracle drugs; this evaluation was tempered only by the toxic effects. I[1] had the dubious honor to be the first in the United States to report a clinical case of concretion formation in the urinary tract, following laboratory observations of concretions in the rat that formed complete molds of ureters and bladder after the administration of sulfapyridine. Neurological manifestations from the use of sulfamethathiazole, resulting in foot and wrist drop, were observed in a fellow physician; the condition persisted for six months.

Although these hazards were minimized by the chemists through the elimination and change of certain radicals, the sulfonamides were subsequently overshadowed by the discovery of the antibiotics. These substances in turn have encountered side reactions to such an extent and of such serious degree as to temper the early enthusiasm for this newer form of therapy.

This situation justifies a new look at the sulfonamides.

My experience, and a review of other clinical reports, indicates that, because of moderate side reactions, low cost, and good tolerance, the more recent compounds, such as Gantrisin, Elkosin, Thiosulfil, and Kynex, are preferable in the acute cases where the infecting organism has not been identified. The sulfonamides are also especially useful in chronic cases where the organisms are harbored in glandular organs, such as in prostatitis, vesiculitis, epididymitis, and chronic pyelonephritis. My *in vitro* and *in vivo* studies of sulfathiazole indicated bacteriostatic effect on the *Staphylococcus*, *Streptococcus*, and gonococcus, but definitely less effect in the bacillary infections.

However, the noteworthy observation in our study of the later sulfonamide, Gantrisin, was its effectiveness on a bacillary organism, *Proteus*, as well as in coccal infections. This was important in the treatment of urinary infections, since *Proteus* splits the urea in the urine and causes a constant alkaline condition that makes methenamine and mandelic acids ineffective and also causes stone formation in the kidney and bladder and encrustations in the retention-draining catheters.[2] The *Proteus* was found to be resistant to most of the drugs then available. Our studies showed penicillin to be totally ineffective, and

TABLE 1
In Vitro SENSITIVITIES OF 1000 ORGANISMS

Organism	No.	Aureomycin		Terramycin		Chloromycetin		Gantrisin	
		Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant
Escherichia coli	245	201	10	156	12	195	11	87	72
Escherichia intermedium	59	51	2	24	21	39	9	12	34
Aerobacter aerogenes	122	97	13	61	17	40	73	6	64
Paracolobactrum coliforme	31	26	4	13	4	15	11	13	7
Pseudomonas aeruginosa	159	43	95	40	64	14	124	6	85
Proteus vulgaris	117	7	102	4	73	86	26	58	26
Alcaligenes fecalis	38	30	2	25	2	17	15	9	22
Staphylococcus	100	60	10	43	35	60	9	18	31
Streptococcus fecalis	81	77	0	44	21	56	10	10	42
Fungus infections	48								
Total	1000								

streptomycin and the tetracyclines were only moderately effective. Our study[3] of the sensitivity of 1000 organisms is shown in TABLE 1.

Subsequent experience with the other sulfonamides follows the same general pattern. The *Pseudomonas* and *Streptococcus fecalis* remain resistant to practically all of the sulfonamides.

Combination of Drug Therapy

Our chief interest in combinations of drugs has been a study of Urosulfin and Azo Gantrisin. The combination with Pyridium definitely lessened the burning and painful urination in acute infections before the organisms were inhibited, and it was useful also for administration before instrumentation in order to alleviate the pain and possible flareup of infection following such procedures.[4] An experimental product chemically combining a sulfonamide with Pyridium was found to cause hepatitis in two patients (J. W. Draper, New York, N. Y.[5]) and in one patient treated by us. Large doses of Pyridium have been known to cause hepatitis; since this experimental drug required such large doses to provide adequate sulfonamide, its manufacture was discontinued.

The most recent combination we have been studying with promising results is oleandomycin with Gantrisin (Gantrimycin).

Thus far my discussion has been confined to the susceptibility of organisms to drugs. From a clinical viewpoint, I propose to discuss the application and value of the sulfonamides as related to specific urogenital organs.

At the outset I must emphasize that the persistence and recurrence of infection are due to failure of drainage or to the presence of obstructive lesions, and all permanent relief depends on finding and removing this obstruction. Such obstructions as stones, prostatic hyperplasia, and strictures are readily recognized and removed, but my attention has been concentrated recently on infections found in the glandular organs: namely, the prostate, the seminal vesicles, the epididymis, and the parenchyma of the kidney.

TABLE 2

INFECTIONS IN 382 CASES OF PYELONEPHRITIS

	Percentages
E. coli	47
Staphylococcus albus	17
Proteus	12
Staph. aureus	6
Nonhemolytic *Streptococcus*	4.5
Pyocyaneus	3
Hemolytic *Streptococcus*	0.5
Mixed	10.0

TABLE 3

NUMBER OF CASES AND ORGANISMS CULTURED IN PYELONEPHRITIS

	Acute pyelonephritis	Chronic pyelonephritis	Total
Number of cases	72	52	124
Number with positive culture	55	39	94
E. coli	48	35	83
Proteus	9	9	18
Hemolytic *Staph. aureus*	7	3	10
Pyocyaneus	5	2	7
Strep. fecalis	3	2	5
Staph. albus	3	0	3
Aerogenes	0	4	4

Complications: 7 cases with prostatitis, 18 cases with calculus, and 4 cases with diabetes.

In chronic pyelonephritis an obstruction in the tubular structures below the lesion often is not demonstrable. It is my opinion that multiple minute infected areas are transformed into colonies of organisms surrounded by fibrous tissue that makes them inaccessible to the drug. In such lesions, prolonged continuous therapy has been applied. I have given Gantrisin over periods of as much as three months with favorable effect on the disease and no deleterious effect on the patient. Gantrisin and Urosulfin were used, since they are excreted quite rapidly in high concentration by the kidneys. Sulfamerazine and Kynex may not be as effective in this condition because of their relatively slow rate of excretion.

In 382 patients with pyelonephritis, C. W. G. Lee[6] isolated the infecting organisms shown in TABLE 2.

Ralph Reader[7] reported his results in acute and chronic pyelonephritis, summarized in TABLE 3. Thus, it is evident that the organisms seen most frequently are sensitive to the sulfonamides.

Chronic Prostatitis and Seminal Vesiculitis

The treatment of these entities by drugs has been generally discouraging. Some have questioned the presence of organisms as the causative factor of pus in the secretion, and others have questioned the presence of the drug in the gland during therapy. Ghormley *et al.*[8] demonstrated that, on culture, 91 per cent showed organisms in prostatic fluid. The pathogens were *Escherichia coli*, *Streptococcus fecalis*, *Aerobacter aerogenes*, *Micrococcus pyogenes*, and *Pseudomonas.*

Walter A. Schloss[9] reported that in cultures of prostatic secretion in 111 cases, 37 per cent showed no growth. *Staphylococcus* was identified as a pure culture in 35 per cent. More than half of the 16 per cent that were mixed infections consisted of combinations of *Staphylococcus* and *Streptococcus*. Thus, it would appear that many of the organisms found on culture in prostatitis can be inhibited by the sulfonamides.

Excretion of Drugs in the Prostatic Secretion

The best evidence of excretion of drugs by the prostate gland was found in a study by James I. Farrell,[10] who demonstrated the presence of such drugs as Mercurochrome, Mapharsen, Pyridium, mandelic acid, and many others.

In my own study, using a dosage of 4 gm. of Azo Gantrisin (or Urosulfin) daily for 5 days, 2.5 mg. per cent of the sulfa drugs were found in an average of 2 ml. of prostatic secretion (pilocarpine was administered just before prostatic massage and collection). In one sample of 0.2 ml. the Gantrisin concentration was 16.2 mg. per cent.

Of 10 patients receiving Azo Gantrisin for 1 month, with weekly prostatic massage, 8 have shown a diminution in the amount of pus to 10 to 15 cells per high-power field.

I have made no study of the effect in epididymitis, and I have been unable to find any such study in the recent literature. I am now beginning to take a new look at the possibilities of prolonged sulfonamide therapy in these glandular urogenital organs, and I intend to extend these studies.

Summary

The new sulfonamides and their combinations with other agents are preferred in the management of acute urinary infections before the infecting organism can be identified. Their value in the treatment of pyelonephritis has been established. Our experience with Azo Gantrisin in prostatitis has been encouraging, especially since we have been able to show the presence of the drug in prostatic secretion. Use of these agents in the treatment of prostatitis, seminal vesiculitis, and epididymitis deserves further study.

Gantrisin or Urosulfin is useful in the treatment of urinary infections caused by the *Proteus* organism. Prolonged moderate doses of these drugs are desirable for patients with indwelling catheters, since it tends to prevent the formation of encrustations.

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CLINICAL USE OF A LIPID EMULSION OF SULFISOXAZOLE

By Saul Krugman

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In recent years the sulfonamides have begun to emerge from the shadow cast upon them by the advent of the newer antimicrobial agents. This emergence has been accelerated by at least three factors: (1) the development of the soluble sulfonamides; (2) the accumulation of data pertaining to the relative therapeutic efficiency of the sulfonamides and the newer agents; and (3) the increasing awareness of the potential side effects of the more recently developed antibacterial drugs.

The soluble sulfonamides have been widely used for approximately ten years. During this period of time these preparations have proved to be relatively nontoxic. The hazard of renal toxicity, particularly tubular obstruction due to precipitation of crystals, has been significantly decreased. These agents have proved to be not only safe, but also comparable to sulfadiazine in therapeutic effect.

The sulfonamides are still the chemotherapeutic agents of choice in the treatment and prevention of meningococcal infections. They are also useful and effective in the management of pneumococcic pneumonia and urinary tract infections caused by coliform bacilli. This effect against both Gram-negative and Gram-positive cocci as well as against Gram-negative bacilli illustrates the broad spectrum effect of these agents. In addition, the sulfonamides are also valuable for the combined therapy of certain types of meningitis. They are usually added to penicillin in the treatment of pneumococcal meningitis and to chloramphenicol for treatment of *Hemophilus influenzae* meningitis.

The increasing awareness of the side effects of many of the newer antimicrobial agents has removed some of the early glamour of these preparations and has placed the sulfonamides in a more favorable light. Hypersensitivity reactions to penicillin, such as urticaria, serum sickness, or even anaphylactic shock, deserve serious consideration. Therapy with the tetracyclines, chloramphenicol, and erythromycin may be complicated by gastrointestinal side effects (nausea, vomiting, or diarrhea) and superinfections. Parenteral streptomycin and dihydrostreptomycin treatment may be followed by damage to the vestibular apparatus or to the eighth nerve, with subsequent deafness. Finally, a significant side effect of the newer antimicrobial agents is the high cost that frequently provokes a significant financial reaction in some patients. These agents cost approximately four to five times as much as sulfonamides.

Sulfisoxazole, a soluble sulfonamide, has been used on our pediatric service for approximately eight years. In our experience this drug has been relatively nontoxic and has compared very favorably to sulfadiazine. The original sulfisoxazole suspension had a bitter taste that was difficult to mask. Subsequently, it was replaced by an acetyl sulfisoxazole preparation that proved to be more palatable. The acetyl sulfisoxazole appears in the blood and body

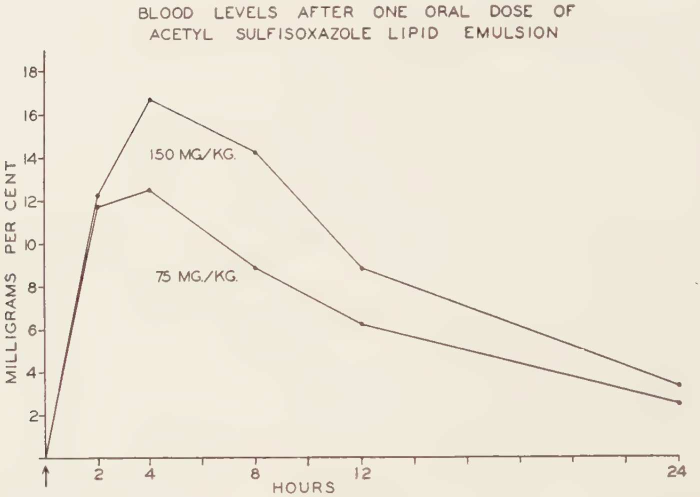

FIGURE 1. Blood levels of free sulfisoxazole in a group of 12 children, 6 for each dosage schedule.[2]

fluids as sulfisoxazole, the conversion presumably occurring in the gastrointestinal tract. Studies reported by Yow[1] indicated that an aqueous suspension of acetyl sulfisoxazole was not absorbed as well as a mixture of sulfadiazine-sulfamethazine-sulfamerazine, or sulfisoxazole or sulfadiazine. Recently, however, my associates and I[2] had an opportunity to study a lipid emulsion of acetyl sulfisoxazole, which produced not only higher, but more prolonged blood levels than the aqueous suspension.

Blood Level Determinations

The acetyl sulfisoxazole in a highly emulsified vegetable oil base* was administered to 69 infants and children 3 mo. to 12 yr. of age. The average age for the group was 2.7 yr. The preparation was available in 2 concentrations: (1) a 10 per cent suspension with 5 cc. equivalent to 0.5 gm. of sulfisoxazole in the N_1-acetyl form, and (2) a 20 per cent suspension with 5 cc. equivalent to 1 gm. Both concentrations were equally effective, but the higher one was easier to administer because of its smaller volume.

Six patients were placed on a single dose schedule of 75 mg. per kg. of body weight; and 6 were given 150 mg. per kg. The average blood levels of free sulfisoxazole for these 2 groups are shown in FIGURE 1. The mean blood levels in mg. per cent on the smaller dosage were 11.8, 12.6, and 6.2 at 2, 4, and 12 hr., respectively; with the higher dose of 150 mg. per kg. the mean blood levels in mg. per cent for these time periods were 12.3, 16.6, and 8.8.

Fifty patients received the acetyl sulfisoxazole lipid emulsion in a dose of 100 mg. per kg. every 12 hr. The blood levels obtained on this multiple dose schedule are illustrated in FIGURE 2. The height of the rectangular bars indicates the average blood levels over a 24-hr. period. The 200 determinations

* Lipo Gantrisin Acetyl, Hoffmann-LaRoche Inc., Nutley, N. J.

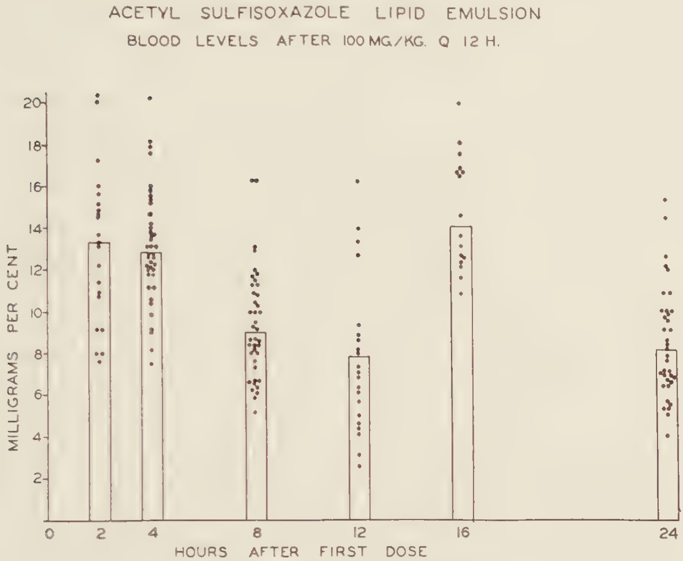

FIGURE 2. Blood levels of free sulfisoxazole on a multiple dose schedule, 100 mg. per kg. of weight every 12 hr. Each dot represents one determination. The top of the bar represents the average level.[2]

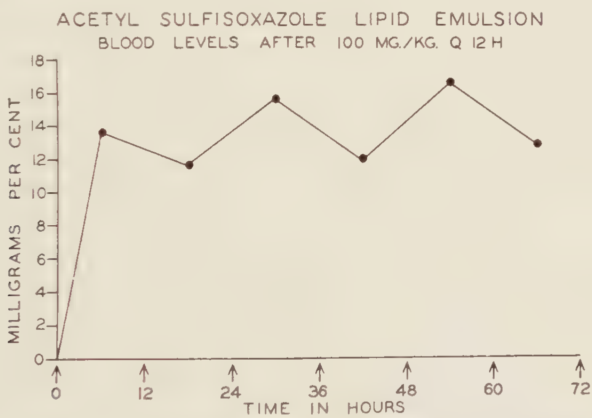

FIGURE 3. Blood levels of free sulfisoxazole on a multiple dose schedule. The arrows indicate the times of drug administration.

are plotted as black dots that illustrate the range in blood levels for each sample period. The mean levels ranged between a high of 16.6 and a low of 8.8 mg. per cent.

Two additional patients were given the emulsion for a 3-day period in a dose of 100 mg. per kg. repeated every 12 hr. The mean blood levels ranged between 11.7 and 16.8 mg. per cent, as shown in FIGURE 3.

The lipid emulsion of acetyl sulfisoxazole had a vanilla mint flavor that was well tolerated by the children. No untoward effects were observed in this

small group of patients. In a group of 142 children studied by Carter,[3] 3 patients (2.1 per cent) developed skin eruptions.

Clinical Evaluation

The adequate evaluation of a chemotherapeutic agent requires data derived from well-controlled observations. As yet, no such study of acetyl sulfisoxazole lipid emulsion has been reported. On the basis of a very limited clinical experience, we have gained the impression that this preparation is at least comparable to the other sulfonamides.

During the past month we have had an opportunity to compare the effect of sulfadiazine and sulfisoxazole on the course of meningococcemia in 2 infants. The clinical picture of both patients is illustrated in FIGURE 4. Patients A. Z. and M. T. had strikingly similar histories and physical findings. Each had had a fever for 12 to 18 hr. and a rash of 6-hr. duration at the time of admission. The extent of their maculopapular, petechial, and purpuric eruptions was comparable. The blood cultures of both patients were positive for type 2 meningococci; only M. T. had a positive cerebrospinal fluid culture. Patient A. T. received sulfadiazine, 200 mg. per kg., intravenously for the first day, followed by the same dosage orally thereafter. In addition he received 1 dose of 600,000 units of aqueous crystalline penicillin. Patient M. T. received sulfisoxazole in the form of its diethanolamine salt intravenously in a dose of 200 mg. per kg. for the first day. Subsequently the lipid emulsion of acetyl sulfisoxazole was given by mouth in a dose of 100 mg. per kg. every 12 hr.

The clinical response to therapy was strikingly similar in both patients. The temperature subsided within 24 hr., the rash disappeared by 36 hr., and both infants had an uneventful recovery. The sulfadiazine-treated infant's course was complicated by a transient gross hematuria and crystalluria that cleared in 3 days.

SULFONAMIDE THERAPY OF MENINGOCOCCEMIA

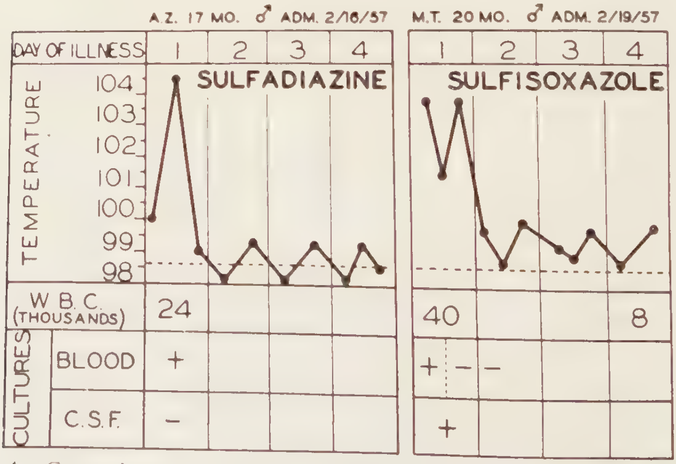

FIGURE 4. Comparison of the effect of sulfadiazine and sulfisoxazole on the course of meningococcemia.

Comment

The rationale for the suspension of a sulfonamide in a lipid emulsion stems from a report by Feinstone and his associates.[4] These investigators presented an abstract of their work at the forty-first general meeting of the Society of American Bacteriologists held at New Haven, Conn., on December 28, 1939. They described the treatment of experimental infections in mice with sulfonamides, comparing the effect of two suspensions: acacia and olive oil. The oily suspension proved to be superior, as indicated by an enhanced therapeutic effect and an increased absorption (higher blood levels). These observations were confirmed by Stephens and Henrickson[5] in 1955. They extended their studies to patients in whom they demonstrated therapeutic blood levels following a twice daily dosage of an oral sulfonamide in a fat emulsion. Svenson *et al.*[6] demonstrated that the oral absorption of acetyl sulfisoxazole, when compared with the aqueous preparation, was greater with the lipid emulsion.

In 1942 Peterson and Finland[7] studied the effect of food on the absorption and excretion of sulfonamide drugs. They demonstrated more complete absorption in subjects given sulfadiazine after the morning meal. The blood levels were consistently higher in this group than in those who received the drug on an empty stomach. In 1945 Reinhold and his associates[8] reported similar findings. The serum sulfadiazine concentration was lower in their fasting subjects than in those who had eaten. It is conceivable that the fat content of the food ingested may have been responsible for the increased absorption of the sulfonamides.

Summary

Our observations have confirmed previously reported studies by others[3, 4] who have demonstrated an enhanced absorption and prolonged effective blood levels following administration of sulfonamides in a lipid emulsion. Acetyl sulfisoxazole lipid emulsion on a 12-hr. dose schedule yielded significant blood levels and was well tolerated.

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CLINICAL USE OF A SULFISOXAZOLE EMULSION

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In his article, Krugman has shown that sustained release of N_1 acetyl sulfisoxazole by suspension in a highly emulsified vegetable oil can produce adequate sulfonamide blood levels over a prolonged period of time. I should like to present a preliminary report on clinical experience with this drug.

To overcome an infection, one must divide therapy into two separate but integrated phases. First, there is the treatment phase (the acutal management of the infection), the elimination of any symptoms or overt manifestation of the disease. Second, there is that period during which therapy is continued beyond the initial stage mentioned, to prevent a recurrence of the disease process. It is during this time that most patients tend to discontinue therapy, either because symptoms have subsided or because continuing treatment of an asymptomatic subject imposes hardships. Sustained release medications make the control of this period easier. In pediatrics, for instance, the children can return to school; in the case of a working mother, she can return her children to custodial care while insuring continued adequate medication.

Method of Study

This study was carried out both in private practice and on the Pediatric Service at the Rochester General Hospital. In private practice the drug was used as one might use any chemotherapeutic or antimicrobial agent. Diagnosis and evaluation were clinical. Criteria as to whether the invading agent was bacterial or viral in etiology were similar to those in which other drugs were used. Since most cases were diagnosed on house calls, few laboratory data were obtained. For instance, in 80 cases of otitis media treated up to March 1, 1957, only 20 cultures were obtained. However, similar criteria are utilized for the evaluation of any therapeutic agent in private practice. Also, nose and throat cultures do not necessarily reflect the true pathogen of otitis media. In the present series, two of the cases showed a discrepancy between the organisms cultured from the middle ear and those from the nose and throat. Results were judged clinically.

The second area of study was at the Rochester General Hospital. This series is still small, with several criteria that must be met before inclusion in this study. Success here is comparable to that in private practice.

The criteria for including patients in the second category included the following requirements:

(1) The leukocyte count must have totaled more than 10,000, with the added evidence of bacterial invasion such as a shift toward the immature forms of the polymorphonuclear leukocytes.

(2) Cultures of the affected areas must have been obtained in all cases before, and again during or after, therapy.

(3) No other chemotherapeutic or antimicrobial agent was to be used at this time.

(4) No previous therapy of a definitive nature could have been used unless it had failed.

(5) Since more than 80 per cent of the hospital cases were private patients, they could be included in the series only with specific permission of the private physician attending the case.

In both areas of the study, if cultures revealed the presence of β-hemolytic *Streptococcus*, Lipo Gantrisin therapy was discontinued and penicillin therapy was substituted, even in cases where some clinical improvement had been obtained on the sulfonamide regime. Recent work with penicillin has shown that it is the drug of choice in β-hemolytic *Streptococcus* infections and that proper management with this drug may prevent the several complications of streptococcal infections. Such cases, however, were not included in this study as failures of Lipo Gantrisin; rather, they were simply eliminated from the study.

Results

The results are presented in TABLES 1, 2, and 3, summarizing about 2 months' experience in private practice and with hospital patients. In the left-hand column are listed the several disease entities. It is important to note that the total number of infections treated during this period amounted to about 260; therefore, the infections treated with the lipid emulsion of sulfisoxazole amounted to 70 per cent of all infections seen in private practice. As a continuing study, a higher percentage of infections seen recently have received Lipo Gantrisin with an equally high percentage rate of cure. Although still small, the series is significant.

An attempt was made to find a common cause for the 7-per-cent failure in the treatment of otitis media. The presence of profuse purulent aural drainage was a factor in 2 cases, but other cases with such drainage were successfully treated. Involvement of the mastoid process clinically and by X ray was a factor in one case (a 7-month-old infant), but other agents were equally unsuccessful until a mastoidectomy was performed. Intolerance of the drug,

Table 1
Lipo Gantrisin Study (Preliminary Report): Private Practice, 176 Cases

Diagnosis	No. cases	No. success	No. failures	Remarks
Otitis media	82	76	6	Failures: (a) pus, (b) mastoid, and (c) intolerance
Tonsillitis	15	15	0	
Tonsillitis with cervical adenitis	12	11	1	Failure on all oral therapy
Pyuria	14	13	1	G.U. anomalies
Lower respiratory infections	43	38	5	Three pneumonia therapies successful
Sinusitis	6	6	0	
Other	4	4	0	G. I. abscess with *Staph. aureus*
Total	176	163	13	

Infections treated during this period: 260.

Table 2
Lipo Gantrisin Study (Preliminary Report): Hospital, 30 Cases

Diagnosis	No. cases	No. success	No. failures	Remarks
Otitis media	12	12	0	
Tonsillitis	1	1	0	
Tonsillitis with cervical adenitis	1	1	0	*Strep. viridans*
Pharyngitis	8	8	0	
Pyuria	3	3	0	
Pneumonia	5	5	0	*Staph. aureus* 3 *H. influenzae* 1
Total	30	30	0	

Table 3
Lipo Gantrisin Study (Preliminary Report): Bacteriological Analysis, 30 Cases

Predominant organism cultured		Comment	
Pneumococci	6		
Bacillus coli	6	U.R.I. infant	2
		Ear	1
Bacillus Proteus	1		
Staph. aureus hem	8	Tetracycline failure	2
H. influenzae	2	Penicillin failure	1
Staph. albus hem	1		
Strep. viridans	4		
Pneumococci plus *H. influenzae*	2		
Failures	1	Draining wound abscess	

mainly on the part of the child who, because of gastrointestinal rebellion, either would not or could not take it, accounted for failure in 2 instances.

Failure in tonsillitis with cervical adenitis was an unusual case. This 4-year-old child was not helped by any of several antibiotics, and so was hospitalized. He developed stertorous respirations and retractions of the thoracic cage. Physical examination revealed marked cervical adenitis and hypertrophied tonsils covered with a purulent exudate. Because of progression of the obstructive respiratory symptoms, tracheotomy was required. The larynx was never visualized, so we cannot be certain that the symptomatology was due to the tonsils. This child subsequently suffered a bout of otitis media with purulent drainage from one ear, which responded satisfactorily to the lipid emulsion of acetyl sulfisoxazole.

It is in the field of lower respiratory infections that the pediatrician finds himself least armed with convincing evidence that any therapeutic agent really benefits his patients. The presence of a lower respiratory infection is usually heralded by a cough—a symptom unique in medicine because of its nuisance value, not only to the patient but also to those around him. Scientific criteria of evaluation break down rapidly in the presense of a cough. Therefore, in this group with 11 per cent failure, one might question the indication for ther-

apy rather than its failure. Among the 43 cases there were 3 instances of pneumonia confirmed by X ray and by cytological and bacteriological tests, all with rapid successful resolution. Perhaps some of the remaining 38 might have shown similar pathology.

Other infections included gastroenteritis, a gluteal abscess with surrounding cellulitis, and evidence of systemic involvement due to *Staphylococcus aureus* hemolyticus. Response was rapid and satisfactory.

TABLE 2 represents the hospitalized cases. Criteria for these patients already have been discussed. They are grouped in the same way that those in private practice have been grouped. The 5 cases of pneumonia are of interest in that 3 were believed due to *Staphylococcus aureus* hemolyticus and 1 due to *Hemophilus influenzae* (unsuccessfully treated by penicillin at home).

TABLE 3 is an analysis of the bacteriology of the 30 hospital cases. All organisms were satisfactorily controlled symptomatically by the lipid emulsion of acetyl sulfisoxazole. Follow-up cultures on these cases have shown either an elimination of the organism or a resumption of the bacterial normal flora as in the case of a respiratory infection where a pure culture of *Staphylococcus aureus* hemolyticus was obtained at the height of the disease process.

Thus, this report has shown encouraging results that accentuate Krugman's work. Lipo Gantrisin is a sulfonamide compound whose properties of sustained release seem to provide chemically effective blood levels for the control of many bacterial infections.

THE CONCURRENT USE OF SULFONAMIDES AND ANTIBIOTICS IN THE TREATMENT OF INFECTIONS: *IN VIVO* AND *IN VITRO* STUDIES OF THE EFFECT OF SULFONAMIDE-ANTIBIOTIC COMBINATIONS ON THE EMERGENCE OF DRUG RESISTANCE*

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The sulfonamides were the first antimicrobial agents administered in combination with antibiotics for the treatment of infection. When penicillin and streptomycin first became available they were often given in combination with the sulfonamide compound in situations in which, by themselves, they failed to produce a dramatic response. Thus, pneumococcal meningitis was treated with penicillin and a sulfonamide, acute brucellosis with streptomycin and sulfadiazine, and urinary tract infections with penicillin or streptomycin and a sulfonamide. In some cases, the use of such combinations was based on *in vitro* observations suggesting an additive or even synergistic activity.[1-4] In others, it was predicated on the unproved premise that a broader "spectrum" of antibacterial activity resulted.

As more potent antimicrobial agents have been developed, the use of antibiotic-sulfonamide combinations has decreased sharply, and at present this form of therapy is mainly of no more than historical interest. There still remain, however, a few situations in which clinical experience suggests that a sulfonamide plus an antibiotic agent produce better therapeutic results than either alone. In some of these the need for the sulfonamide is questionable. In others, combined treatment is regarded by some investigators as the procedure of choice. Although *Nocardia* infections respond to chlortetracycline, penicillin, or streptomycin, it has been recommended that antibiotics effective *in vitro* should be added to, rather than substituted for, the sulfonamides.[5] Actinomycosis may be treated successfully with either penicillin or sulfadiazine, although the combination of drugs is preferred by some[6, 7] because of the impression of more rapid response and smaller risk of relapse. Sulfadiazine or sulfisoxazole (Gantrisin) is highly effective in mild to moderately severe meningococcal meningitis. When the disease is overwhelming, and especially when the possibility of the Waterhouse-Friderichsen syndrome is suspected, however, the simultaneous use of large doses of penicillin and sulfadiazine has been strongly urged.[8] The therapy of choice for pneumococcal meningitis in some clinics is sulfadiazine or sulfisoxazole plus penicillin, given intrathecally as well as intramuscularly, because this combination is thought to yield a higher survival rate than massive doses of the antibiotic alone.[9] Sulfonamides are very often included in chemoprophylactic programs employed for preparation of the bowel for surgery; the most commonly used drugs,

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sulfasuxidine, sulfathalidine, and phthalylsulfacetamide, are administered together with a variety of antibiotic agents.[10, 11] Combined sulfonamide-antibiotic therapy appears to be the method of choice in the management of *Hemophilus influenzae* meningitis; chloramphenicol plus sulfadiazine, or streptomycin (intrathecal and intramuscular injection) plus sulfadiazine or sulfisoxazole produce better therapeutic results than single antimicrobial agents.[9, 12-15]

It has been suggested that combining sulfonamides with antibiotic agents may delay the emergence of drug resistance in some organisms. Carpenter and his co-workers[16] noted that the addition of sulfathiazole suppressed the development of insensitivity of the gonococcus to penicillin. Sulfadiazine was reported by Pulaski and Baker[17] to inhibit the appearance of streptomycin resistance in *Escherichia coli*, and by Klein and Kimmelman[18] to maintain the sensitivity of *Staphylococcus aureus* to penicillin and streptomycin.

The purpose of this paper is to present data indicating that the emergence of resistance of some bacteria to antibiotics can be suppressed by the addition of sulfonamides. Under certain conditions this phenomenon is readily demonstrable *in vitro*. Evidence will be offered to suggest that it also occurs *in vivo*.

Clinical Studies

H. influenzae meningitis. Twenty-two patients, aged 6 mo. to 3 yr., with type B *H. influenzae* meningitis were treated with 2 to 3 intrathecal injections of 15 to 25 mg. each of streptomycin, and approximately 25 mg. per kg. per day of the same drug given in divided doses intramuscularly. Sensitivity studies of the organisms isolated from the spinal fluid or blood, or both, in all cases prior to the initiation of therapy revealed that they were inhibited by 2.5 to 5 μg. of streptomycin per ml. Treatment produced a reduction of the number of cells and an increase in the sugar content in the spinal fluid in all of the patients within 36 hr. after it was started; organisms were present in only very small numbers in a few children, but could not be demonstrated by culture in the majority. Eighteen patients recovered completely. In 4, however, the meningitis relapsed in 72 to 96 hr.; this was confirmed by the reappearance of purulent spinal fluid from which *H. influenzae* (type B) was isolated in large numbers. These organisms were found to be insensitive to 1000 μg. of streptomycin per ml. The administration of sulfadiazine and type-specific antiserum produced recovery in 2 children; the other 2 succumbed to the disease.

Forty young patients with *H. influenzae* meningitis were treated with streptomycin in the same manner as those described above, but were given, in addition, sulfadiazine or sulfisoxazole (0.1 gm. per pound per day, half of of the total calculated quantity constituting the initial dose). Sensitivity tests of many of the isolated strains revealed that they were highly susceptible to streptomycin. Studies of sulfonamide sensitivity were not carried out. *H. influenzae* could not be recovered from the spinal fluid in any patient later than 24 hr. after the initiation of combined therapy. Complete recovery, without relapse or complication, occurred in every instance.

Pseudomonas aeruginosa meningitis. A 16-yr.-old boy developed meningitis due to *Ps. aeruginosa* following spinal anesthesia. Prior to the initiation of treatment, the organism was inhibited by 7.8 μg. of streptomycin per ml. and 25 mg. per 100 ml. of sulfadiazine. Therapy consisted of the daily administration of 100 mg. of streptomycin intrathecally, 4 gm. of the antibiotic in divided doses intramuscularly, and full oral doses of sulfadiazine; the sulfonamide blood level ranged between 9 and 13 mg. per 100 ml. The drugs were discontinued after 7 days; clinical and bacteriological relapse occurred 4 days later. The strain of *Ps. aeruginosa* isolated from the spinal fluid at this time was sensitive to 15 μg. of streptomycin per ml. and to 25 mg. of sulfadiazine per 100 ml. The same type of therapy was reinstituted for 9 days and was followed by marked improvement. Within a few days after treatment was stopped, however, the disease recrudesced, and organisms again were recovered from the spinal fluid. The sensitivity of the bacteria to both streptomycin and sulfadiazine was unchanged. Three more relapses occurred. In none of these was the *Pseudomonas* resistant to either antimicrobial agent. Recovery was finally accomplished when combined streptomycin-sulfadiazine administration was continued for 4 wk., the dose of sulfonamide being increased to 12 gm. per day in order to maintain a spinal-fluid level greater than 25 mg. per 100 ml.

Another patient with *Ps. aeruginosa* meningitis following spinal anesthesia was a 58-yr.-old woman. The organism recovered from her spinal fluid was inhibited by 30 μg. of streptomycin, and more than 5 but less than 25 mg. of sulfadiazine per 100 ml. Treatment was the same as that in the patient described above and was continued for 18 days. Thirty-two days after recovery, manifestations of meningitis reappeared. *Ps. aeruginosa* was again isolated from purulent spinal fluid. The sensitivity of the strain isolated at this time was identical with that of the organism recovered when the disease first developed. Retreatment with the initial combined drug regimen for 4 wk. was followed by complete recovery.

These observations suggested that combined sulfonamide-antibiotic treatment suppressed the emergence of streptomycin resistance in *H. influenzae* and *Ps. aeruginosa in vivo.* This was striking because of the ease with which both organisms develop drug insensitivity when exposed to streptomycin alone, both *in vitro* and *in vivo.* These clinical experiences prompted a study of the effect of various antibiotic-sulfonamide mixtures on the emergence of drug resistance *in vitro.*

Experimental Studies

Methods

Several strains each of *Staph. aureus* and *E. coli* were studied. Each organism was tested for sensitivity to antibiotics, sulfadiazine, and sulfisoxazole by the test tube dilution method. The medium described by Strauss *et al.*,[19] in the form of broth or agar, was used throughout the study because it contained a minimum of inhibitory substances. In some instances it was necessary to resort to repeated subculture in order to acclimatize some of the bacteria to the medium. Stock solutions of benzyl penicillin G, streptomycin sulfate,

chloramphenicol, chlortetracycline, and tetracycline were freshly prepared and added to broth in quantities necessary to produce the desired concentrations. The sodium salts of sulfadiazine and sulfisoxazole were employed; the *p*H of the medium was adjusted to 7.4, when necessary, after the addition of varying quantities of the sulfonamides.

The stock solutions of the various antibiotics investigated were made in broth by the addition of concentrations 8 to 10 times greater than those required to inhibit the growth of the organism under study. The sulfonamides were dissolved to their limit of solubility in a liquid medium. The drug-mixture stock solutions contained maximal soluble quantities of sulfadiazine or sulfisoxazole and 8 to 10 times the inhibitory amount of antibiotic. The stock solutions were diluted twofold serially in broth, and 1 ml. of each dilution was added to Petri plates containing 9 ml. of agar. Three sets of plates were used in each step of the experiments; one contained varying quantities of the antibiotic alone, the second sulfonamide alone, and the third the drug combination. Control medium containing no drugs was inoculated in each experiment. The agar containing the single and mixed drugs in varying concentrations was streaked with one loopful (1 mm. in diameter) of inoculum prepared by suspending a 2-mm. loopful of the surface growth of a 24-hr. drug-free agar culture of the organism being studied in 0.2 ml. of broth. All plates were examined after 48 hr. of incubation at 37° C., and subcultures were made from those containing the highest concentration of single or mixed drugs that revealed adequate growth to another set of agar plates to which had been added different quantities of antibiotic or sulfonamide alone or in combination. Subcultures were carried out every 48 hr. for 10 to 20 transfers. At the end of this time the organisms were isolated from the media containing the highest concentration of single or mixed drugs that failed to suppress multiplication, and were tested by the tube-dilution method for sensitivity to the antibiotic and to the sulfonamide.

Results

Penicillin plus sulfadiazine or sulfisoxazole—Staph. aureus. Four strains of *Staph. aureus*, all of which were sensitive to penicillin, but only 2 of which were inhibited by sulfadiazine and sulfisoxazole, were exposed to varying quantities of the single drugs and antibiotic-sulfonamide combinations. After 12 to 18 transfers, all of the organisms cultivated in penicillin, sulfadiazine, or sulfisoxazole alone became resistant to these agents. On the other hand, the strains initially susceptible to the antibiotic and the sulfonamides, when subcultured in media containing a mixture of the two drugs, retained their sensitivity to penicillin but, with one exception, not to the sulfonamide. The organisms originally sensitive to penicillin, but not to sulfadiazine or sulfisoxazole, became resistant to the antibiotic when serially transferred in broth containing drug mixtures (TABLE 1).

Streptomycin plus sulfadiazine or sulfisoxazole—Staph. aureus. Five strains of *Staph. aureus* were studied (TABLE 2). Two were sensitive to sulfadiazine, sulfisoxazole, and streptomycin. One was resistant to the antibiotic, but not to the sulfonamides. Another was inhibited by streptomycin, but not

TABLE 1

EFFECT OF COMBINING PENICILLIN WITH SULFADIAZINE OR GANTRISIN ON EMERGENCE OF RESISTANCE IN *STAPH. AUREUS*

Strain No.	Initial sensitivity			Sensitivity after 12–18 transfers in: single drugs			P + SD		P + SZ	
	P	SD	SZ	P	SD	SZ	P	SD	P	SZ
1	0.12	3.1	12.5	5	>100	>200	0.3	>100	<0.06	25
4	0.03	12.5	25	10	>100	>200	0.12	>100	<0.06	200
7	0.3	>100	200	40	>100	>200	20	>100	50	>200
9	0.3	>100	200	20	>100	>200	20	>100	50	>200

Symbols: P = penicillin, units/ml.; SD = sulfadiazine, mg. per cent; and SZ = sulfisoxazole (Gantrisin), mg. per cent.

by sulfadiazine or sulfisoxazole. Growth of the fifth was not suppressed by any of the antimicrobial agents. The organisms transferred in media containing single drugs became highly resistant after 10 passages. When sulfonamide was mixed with streptomycin, the strains initially sensitive to both drugs developed a moderate loss of sensitivity to the antibiotic, but a loss of much smaller degree than that which followed exposure to streptomycin alone; complete resistance to the sulfonamide developed, however. The organisms originally insensitive to the antibiotic alone, or to the sulfonamide alone, or to both, became more resistant to all of the agents when subcultured repeatedly in media containing sulfonamide-antibiotic mixtures.

Streptomycin plus sulfisoxazole—*E. coli*. Four strains of *E. coli* were exposed to streptomycin and sulfisoxazole alone and in combination. Initial sensitivity determinations revealed that multiplication of all of the organisms was prevented by relatively small quantities of streptomycin; two were susceptible and two resistant to the sulfonamide. After ten passages in media containing single antimicrobial agents, all of the strains developed high-grade insensitivity to the drugs. The same number of passages in antibiotic-sulfonamide mixtures produced a striking suppression of the emergence of resistance to strep-

TABLE 2

EFFECT OF COMBINING STREPTOMYCIN WITH SULFADIAZINE OR GANTRISIN ON EMERGENCE OF RESISTANCE IN *STAPH. AUREUS*

Strain No.	Initial sensitivity			Sensitivity after 10 transfers in: single drugs			SM + SD		SM + SZ	
	SM	SD	SZ	SM	SD	SZ	SM	SD	SM	SZ
3	0.7	6.2	12.5	200 T	>100	>200	25	>100	50	>200
4	0.7	12.5	25	10 T	>100	>200	25	>100	25	>200
1	500	3.1	12.5	200 T	>100	>200	10 T	>100	10 T	>200
8	0.7	>100	200	10 T	>100	>200	10 T	>100	5 T	>200
9	500	>100	200	100 T	>100	>200	25 T	>100	50 T	>200

Symbol: SM = streptomycin, μg./ml.

Table 3

Effect of Combining Streptomycin with Sulfisoxazole on Emergence of Resistance in *E. coli*

Strain No.	Initial sensitivity		Sensitivity after 10 transfers in:			
			single drugs		SM + SZ	
	SM	SZ	SM	SZ	SM	SZ
1	12.5	12.5	200 T	>200	3.1	>200
2	12.5	6.2	100 T	>200	6.2	>200
3	6.2	200	200 T	>200	100 T	>200
4	12.5	200	100 T	>200	50 T	>200

tomycin in the strains originally inhibited by both antibacterial substances, but not in those that initially had been sensitive to the antibiotic, but not to sulfisoxazole (table 3).

Chloramphenicol plus sulfadiazine or sulfisoxazole E. coli. As can be observed in table 4, exposure of four strains of *E. coli* to mixtures of chloramphenicol with sulfadiazine or sulfisoxazole did not suppress the emergence of resistance to the antibiotic, regardless of the initial drug sensitivity. Organisms originally suppressed by all three antimicrobial agents and those inhibited by chloramphenicol, but not by the sulfonamides, developed the same degree of resistance.

Chlortetracycline or tetracycline plus sulfadiazine—Staph. aureus. Four strains of *Staph. aureus*, all of which were sensitive to tetracycline and chlortetracycline, but only two of which were inhibited by small quantities of sulfadiazine, were studied for sensitivity to the antibiotics and the sulfonamide before and after twenty passages in media containing single agents and mixtures of the drugs (table 5). As was noted with the chloramphenicol-sulfadiazine combination, the emergence of resistance to the tetracycline compounds was not suppressed by the addition of sulfadiazine, even when the organisms were susceptible to both antimicrobial agents prior to exposure to drug mixtures.

Table 4

Effect of Combining Chloramphenicol with Sulfadiazine and Sulfisoxazole on Emergence of Resistance in *E. coli*

Strain No.	Initial sensitivity			Sensitivity after 17 transfers in:						
				single drugs			CM + SD		CM + SZ	
	CM	SD	SZ	CM	SD	SZ	CM	SD	CM	SZ
5	12.5	12.5	25	500	>100	>200	500	>100	>500	>200
7	25	6.2	25	>500	>100	>200	>500	>100	>500	>200
9	50	>100	>200	>500	>100	>200	>500	>100	>500	>200
12	25	>100	>200	>500	>100	>200	>500	>100	>500	>200

Symbol: CM = chloramphenicol, μg./ml.

TABLE 5

EFFECT OF COMBINING CHLORTETRACYCLINE OR TETRACYCLINE WITH SULFADIAZINE ON EMERGENCE OF RESISTANCE IN *STAPH. AUREUS*

Strain No.	Initial sensitivity			Sensitivity after 20 transfers in: single drugs			CT + SD		TC + SD	
	CT	TC	SD	CT	TC	SD	CT	SD	TC	SD
21	0.8	1.6	3.1	200	25	>100	100	>100	50	>100
4	3.2	3.2	12.5	100	100	>100	100	>100	100	>100
11	3.2	6.2	>100	50	50	>100	100	>100	50	>100
12	1.6	3.2	>100	200	25	>100	100	>100	25	>100

Symbols: CT = chloretetracycline (Aureomycin), μg./ml.; TC = tetracycline, μg./ml.

DISCUSSION

The clinical usefulness of sulfonamide-antibiotic combinations is limited. While there are some instances in which such mixtures are thought to produce better clinical results than either drug alone, further critical evaluation is required to establish such therapy as essential. The disease that, in our experience, is best managed by the simultaneous administration of a sulfonamide and an antibiotic is *H. influenzae* meningitis. Here, clinical study suggests that the important effect of the addition of sulfadiazine or sulfisoxazole to streptomycin is suppression of the emergence of antibiotic resistance, rather than an additive or synergistic effect, although this may be an added feature. The use of sulfonamide-antibiotic combinations in infections in which they have not been proved by clinical trial to be superior must be approached with a highly critical attitude and with caution, since patients may be exposed to all of the risk of drug reactions with very little, if any, increase in benefit. Although *in vitro* studies suggested that mixing the two types of agents produced greater antimicrobial activity, clinical proof for this was lacking in most situations. The administration of sulfonamides and antibiotics together, merely for the purpose of increasing the number of "shells" in the chemotherapeutic "shotgun" is to be decried.

In vitro studies indicated that the addition of sulfadiazine or sulfisoxazole to penicillin or streptomycin suppressed the emergence of resistance of bacteria to the antibiotics, but not to the sulfonamides, if the organisms were initially sensitive to both agents. If, however, they were not susceptible to one of the drugs in the mixture, the appearance of antibiotic insensitivity was not prevented. With chloramphenicol, chlortetracycline, and tetracycline, the development of resistance could not be inhibited by the addition of a sulfonamide compound, even when the bacteria were initially sensitive to the antibiotics and to sulfadiazine or sulfisoxazole. These observations suggest that the emergence of drug resistance following exposure of organisms to antibiotic-sulfonamide mixtures is related to the mode of action of the antibiotic. If it is bactericidal, the addition of a sulfonamide compound is effective; if it is bacteriostatic, no effect is produced. Two factors limit the application of sulfonamide-antibiotic mixtures for the purpose of

depressing the development of drug insensitivity in clinical infections. First is the fact that a large number of the strains of *E. coli*, *Staph. aureus*, and other organisms responsible for illness are sulfonamide-resistant. Second is the fact that, with the exception of *H. influenzae* and possibly *Ps. aeruginosa* meningitis, proof that the emergence of drug resistance in human infections can be prevented by combined sulfonamide-antibiotic therapy is lacking.

Conclusions

(1) The clinical use of combined sulfonamide-antibiotic therapy appears to be indicated in the treatment of *H. influenzae* meningitis, in which the addition of sulfadiazine or sulfisoxazole (Gantrisin) inhibits the development of streptomycin resistance in the influenza bacillus.

(2) Combinations of a sulfonamide with penicillin or streptomycin suppress the emergence of resistance to these antibiotics *in vitro*; the organisms have to be sensitive initially to both antimicrobial agents.

(3) The addition of a sulfonamide to chloramphenicol, chlortetracycline, or tetracycline does not inhibit the appearance of antibiotic resistance, regardless of the original drug sensitivity of the organisms.

(4) When combined with bactericidal antibiotics, but not when mixed with bacteriostatic agents, sulfonamides prevent the development of drug resistance.

(5) The clinical application of sulfonamide-antibiotic combinations is limited, and has to be restricted to the situations in which proof of their effectiveness is based on critically evaluated experience.

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CLINICAL TOXICITY OF SULFONAMIDES

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Introduction

More than two decades have elapsed since Gerhard Domagk's epochal discovery that, to quote him in translation: "Prontosil enables the mouse infected with streptococci to live if it is treated subcutaneously or orally with the drug within 24 hours of the beginning of the infection."[1] After a slow and little-noticed beginning of successful human application in Germany, spearheaded by the pioneering work of scientists in many other countries, such as the Tréfouëls *et al.*[2] in France, Colebrook and Kenny[3] and Buttle *et al.*[4] in England, and Long and Bliss,[5] Marshall *et al.*,[6] and others in the United States, sulfonamide therapy gained rapid and widespread acceptance throughout the world. The startling and world-shaking realization that now, for the first time in history, systemic bacterial infections could be treated specifically by the administration of chemical compounds, because these substances lacked prohibitive toxicity to the tissues of the host, resulted in a veritable flood of experimental and clinical publications. So thorough and so extensive were these reports (among others[7-13]), that a comprehensive picture of the entire range of clinical toxicity became available within a relatively short time after the introduction of each new sulfonamide compound. For example, in the excellent paper of Van Dyke,[11] presented fourteen years ago, we find little to be modified or to be added to the lucid description of the signs and symptoms of clinical toxicity.

However, while the type and character of toxic reactions have not changed materially from those encountered in the early years of sulfonamide therapy, their incidence has shown a remarkable decline with the decrease in the use of the older and more toxic compounds, with the introduction of better tolerated sulfonamide derivatives of lower over-all toxicity, and with the development of a number of highly effective safety measures. This dramatic drop in the occurrence of potentially dangerous toxic reactions, particularly in renal complications, sensitization reactions, and blood dyscrasias was, in fact, one of the primary reasons for the amazing resurgence of sulfonamide therapy in recent years, which is the more significant since it occurred in the era of antibiotics.

Factors Influencing Toxicity

A number of factors tends to influence both the incidence and the severity of toxic reactions caused by sulfonamides (TABLE 1). It is quite clear that an increase either in duration or dosage, or both, will tend to increase the incidence and severity of many untoward reactions. I shall demonstrate that this applies even to the production of primary sensitization.

Among systemic sulfonamides, the mother compound, sulfanilamide (para-

TABLE 1

FACTORS INFLUENCING INCIDENCE AND SEVERITY OF TOXIC REACTIONS FROM SULFONAMIDES

(1) Duration of administration.
(2) Total dosage (tissue concentration).
(3) Type of heterocyclic ring in N_1 substituted sulfonamide.
(4) Solubility of free acid and N_1 acetylated product in normal *p*H range of human urine.
(5) Pre-existing state of kidney.
(6) Age of patient.
(7) Nutritional status of patient.
(8) Readministration.
(9) Allergic diathesis.

amino benzene sulfonamide), apparently can induce all toxic reactions encountered at the bedside with any one of its N_1-substituted derivatives, with two notable exceptions: only sulfanilamide induces acidosis, because a free $—SO_2NH_2$ group is required for the inhibition of carbonic anhydrase, and sulfanilamide does not cause mechanical blockage of the kidneys by crystalline precipitate, because this compound, as well as its acetylated product, is highly soluble.

Substitution, with heterocyclic rings at the N_1 position of the sulfanilamide molecule, primarily modifies incidence and severity of toxic reactions characteristic for this drug. It usually also substantially decreases solubility. Most heterocyclic derivatives of sulfanilamide therefore may cause mechanical blockage of urinary pathways. Of the heterocyclic rings used in N_1 substitution, the pyridine ring is the least desirable, raising the incidence of nausea and vomiting to as much as 50 per cent and exposing the kidney to the serious danger of crystalline obstruction that cannot be obviated by alkalization. Thiazole, although far better tolerated by the patient and creating somewhat better solubility characteristics for the free acid, is responsible for the highest incidence of sensitization reactions (more than 11 per cent) and, because of the extremely low solubility of acetyl sulfathiazole (6 mg. per cent at 37° C. in water), it is equally dangerous to the kidney. The pyrimidine ring appears to be the most desirable, since it accounts for the excellent over-all tolerance of all sulfapyrimidines. The compounds of this group, now in extensive clinical use, cause little nausea and vomiting or other signs of central nervous system involvement and are distinguished by a remarkably low incidence of allergic reactions. Although sulfadiazine and its monomethyl derivative, sulfamerazine, have unsatisfactory solubility characteristics, far-reaching protection of the kidneys can be accomplished by adequate alkalization of the urine. This is in striking contrast to the ineffectiveness of this measure against urinary concretions from sulfapyridine and sulfathiazole. It is understandable, therefore, why sulfadiazine and its congeners, either singly or in triple mixture, form the backbone of modern sulfonamide therapy. We do not know why sulfathiazole and sulfadiazine cause relatively little nausea and vomiting, while the incidence is so high for sulfapyridine. In this connection it is of interest to observe that both thiazole and pyrimidine are part of the thiamine molecule and thus are, in a sense, "physiological" compounds.

Because of the pre-eminent position of sulfadiazine and its derivatives in modern sulfonamide therapy, and the enormous experience accumulated with

this group of sulfonamides over the last fifteen years of extensive clinical application, sulfadiazine will serve as the standard of comparison in describing type and incidence of reactions. Information on the sulfapyrimidines is derived from a number of reports covering the period from 1942 to 1954.[11-13, 15-19]

No data of equal latitude are available for sulfisoxazole (Gantrisin), the only newer systemic sulfonamide outside the sulfapyrimidine series.* The recent tabulation of Kutscher *et al.*[19] confirms the considerable renal safety of this sulfonamide, but also indicates a sensitization potential in excess of that of sulfadiazine.

Pre-existing renal dysfunction may predispose to mechanical renal damage from sulfonamides and may contribute to toxicity by raising sulfonamide tissue concentrations because of inadequate elimination with the urine.

Adults show greater liability to the development of complications than children, except for acute hemolytic anemia, which is more frequent in children. Also, toxic reactions are more apt to occur in old age and, in general, in patients of poor nutritional status.

Re-administration enhances the chances for the appearance of allergic reactions. Untoward reactions of this type, encountered during the first administration of a sulfonamide, substantially increase the likelihood of a severe toxic response to a subsequent course of the drug. A higher incidence of sensitization reactions should be expected also in patients with a personal or family history of allergic diathesis.

It should be realized that it is sometimes extremely difficult to distinguish the toxic effects of drugs from the signs and symptoms of acute illness, particularly if such remedies are used in serious febrile disease, and that clear-cut differentiation from the effects of the sickness itself may sometimes be impossible. Thus, some toxic drug reactions may be overlooked, whereas manifestation of the disease may be falsely counted as drug reaction. This problem is particularly acute with rare and often bizarre reactions as they appear in single case reports, so that our knowledge of rare complications is still rather fragmentary and unclear. I am afraid that I shall not be able to shed much additional light on them here. A thorough knowledge of the toxic potentialities of sulfonamides is essential, so that serious complications can be recognized early and the administration of the offending drug can be discontinued at once.

Relationship of Toxicity in Animals to Toxic Reactions in Man

The vast clinical experience of twenty years has made it possible to re-evaluate critically some of our early concepts on toxicity and to obtain some more definite answers in dubious situations. It has enabled us also to continue matching clinical data with animal experimental results for the purpose of delineating more precisely the value and the limitations of animal experimental studies in predicting the clinical toxicity of new compounds of this series.

* The sulfapyridazines are not included, since this group of drugs has been introduced at the bedside too recently for consideration here.

It has become abundantly clear that, except for mechanical obstruction of the urinary tract, which can be tested with precision in the experimental animal, toxicity studies in animals are of limited value in predicting the potential clinical toxicity of a new sulfonamide compound. Toxic reactions observed with dosages so excessive as to induce death after a single administration ("acute toxicity") give information on the inherent toxicity of the drug in question concerning primarily the central nervous system. Thus, it can

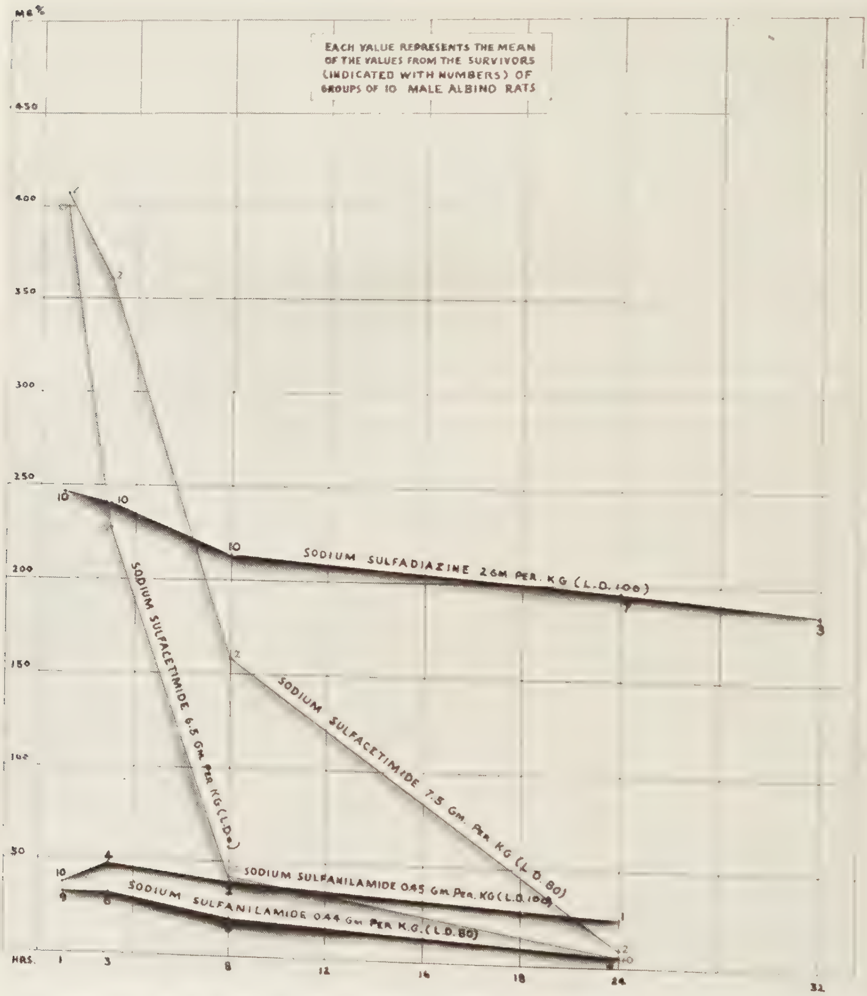

FIGURE 1. Blood levels of sulfacetimide, sulfadiazine, and sulfanilamide after intraperitoneal injection of single lethal dosages of the sodium salts of these three compounds in albino rats. The animals tolerated excessively high sulfacetimide levels (steep drop due to rapid renal elimination), high sulfadiazine levels (long sustained due to renal obstruction by poorly soluble drug precipitate), and only strikingly low sulfanilamide levels (slow drop due to pronounced tubular reabsorption of sulfanilamide). From Lehr. 1945. J. Urol. **54**: 87.

be demonstrated that albino rats tolerate excessively high blood levels of sulfacetimide (250 to 400 mg. per cent) for hours, and survive high sulfadiazine levels (200 to 250 mg. per cent) for days, but succumb invariably and rapidly to concentrations of less than 50 mg. per cent of sulfanilamide (FIGURE 1). These results, while theoretically interesting, may have little meaning at the bedside. The effect of continuous administration or of repeated doses of sulfonamides in moderate amounts over a period of days or weeks (subacute or chronic toxicity) may have a far greater bearing upon possible harmful effects in man. Organic damage observed under these conditions in parenchymatous organs, in the blood, in the bone marrow, or in the central and peripheral nervous system may serve as a timely warning of possible trouble ahead.

On the other hand, the production of complications resembling toxic reactions in human beings that require very special experimental conditions not applicable at the bedside make it appear doubtful whether the experimental mechanism is implicated at all in the clinical picture. Into this category belong, for instance, the interesting studies of Kornberg *et al.*[20] on the production of granulocytopenia and anemia in rats fed sulfonamides in purified diets. On the other hand, the beautiful work of Rich and Gregory[21] on periarteritis nodosa-like lesions induced in rabbits by horse serum and sulfonamides lies well within the realm of clinical possibility.

As regards pharmacological and toxic effects in the experimental animal, the sulfonamides are remarkably inert if the therapeutic dosage range is not exceeded. Therefore, most of the toxic effects observed in man are not reproducible in the experimental animal. This applies in particular to the important group of sensitization reactions. On the other hand, it should be clear, that those reactions, such as renal complications, that can be reproduced should have definite significance, and that others obtained only with excessive dosages may have a meaning if properly interpreted.

Clinical Toxicity

The toxic reactions encountered at the bedside in systemic sulfonamide therapy are listed in order of their frequency and importance in TABLE 2. From this table it can be seen that subgroup A entails those reactions that require watchfulness and caution on the part of the treating physicians. In fact, most manifestations comprised in the enumeration of points 1 to 4 would be handled best by immediate discontinuance of sulfonamide administration

TABLE 2

CLINICAL TOXICITY OF SULFONAMIDES*

(A) *Potentially serious reactions*	(1) Complications of the urinary tract
	(2) Sensitization reactions
	(3) Blood dyscrasias
	(4) Hepatitis
(B) *Milder reactions*	(5) Acidosis
	(6) Cyanosis
	(7) Central and peripheral nervous system reactions

* In order of frequency and importance.

and immediate institution of measures to hasten the elimination of the drug from the body. Because they do not require immediate drug withdrawal, exceptions in group 1 are crystalluria and microscopic hematuria and, in group 3, mild anemia and leukopenia.

Reactions listed under subgroup B are obviously of less importance and, except for rare psychosis, intractable nausea, and vomiting or peripheral neuritis, do not usually call for discontinuation of therapy.

Potentially Serious Reactions

Complications of the Urinary Tract

The most serious and frequent complications from the systemic administration of the sulfonamides are those related to mechanical obstruction of the urinary pathways by crystalline concretions of the sulfonamides. The shape of crystals commonly found in the urine of patients receiving sulfapyridine, sulfathiazole, and sulfadiazine are illustrated in FIGURES 2, 3, and 4. Although this never occurred with sulfanilamide itself, the first two heterocyclic derivatives of this compound, sulfapyridine and sulfathiazole, have caused many cases of serious injury to the kidney and, not infrequently, even death in renal failure.

In the earlier years of sulfonamide therapy the proportionate contribution of various factors to renal injury was not as clearly apparent as it is today. It was accepted from the very beginning that obstruction of renal tubules and ureters with concretions of poorly soluble acetylated metabolites accounted for many complications. However, some patients had died in anuria without ever having shown crystalluria or hematuria, and without revealing any concretions in the urinary tract at autopsy. Hence, a "toxic nephrosis" was believed to be responsible for many deaths. Finally, an allergic type of renal damage was believed to exist.

It is important to point out that, at this writing, modern pharmacology textbooks and monographs still describe the three types of renal damage from sulfonamides as (1) mechanical, (2) nephrotoxic, and (3) allergic, without indicating that mechanical injury is the rule and that primary nephrotoxic, that is, nonobstructive lesions, as well as allergic damage represent very rare exceptions so rare, in fact, that they do not enter significantly into the computation of incidence of renal damage. It seems to me vital to be able to reassure the treating physician that almost complete renal safety can be achieved in sulfonamide therapy by preventing mechanical obstruction a fact that, although overwhelmingly established after twenty years of animal experimental and clinical studies, is probably not sufficiently known or at least not adequately emphasized, and might, in fact, still be unacceptable to some.

The importance of this point calls for detailed presentation of the evidence. In the animal experiment it can be demonstrated that sulfonamides of inadequate solubility, such as sulfapyridine, sulfathiazole, sulfadiazine, sulfamerazine, and sulfapyrazine (TABLE 3), when given in high dosage, will readily form concretions in the urinary pathways, resulting in renal tubular irritation

FIGURE 2. Urinary crystals of acetylsulfapyridine. (*A*) "*Whetstones*": the most common form, varying considerably in size (0.008 by 0.01 mm. to 0.05 by 0.08 mm.), transparent, colorless, and usually with notching or serration of one or both edges. (*B*) "*Arrowheads*" (0.016 by 0.56 to 0.04 by 0.92 mm.): similar to whetstones but wedge shaped, presenting one sharp point and a pronounced sawlike dentification of one or both sides, with a rounded base showing irregular notchings. (*C*) *Centrally waisted sheaves* composed of needlelike crystals, usually rather large (the largest crystals pictured are 1 by 4 mm.). These sheaves may conglomerate and form crosslike and starlike structures. X176. From Lehr & Antopol. 1942. Am. J. Clin. Pathol. **12**: 200.

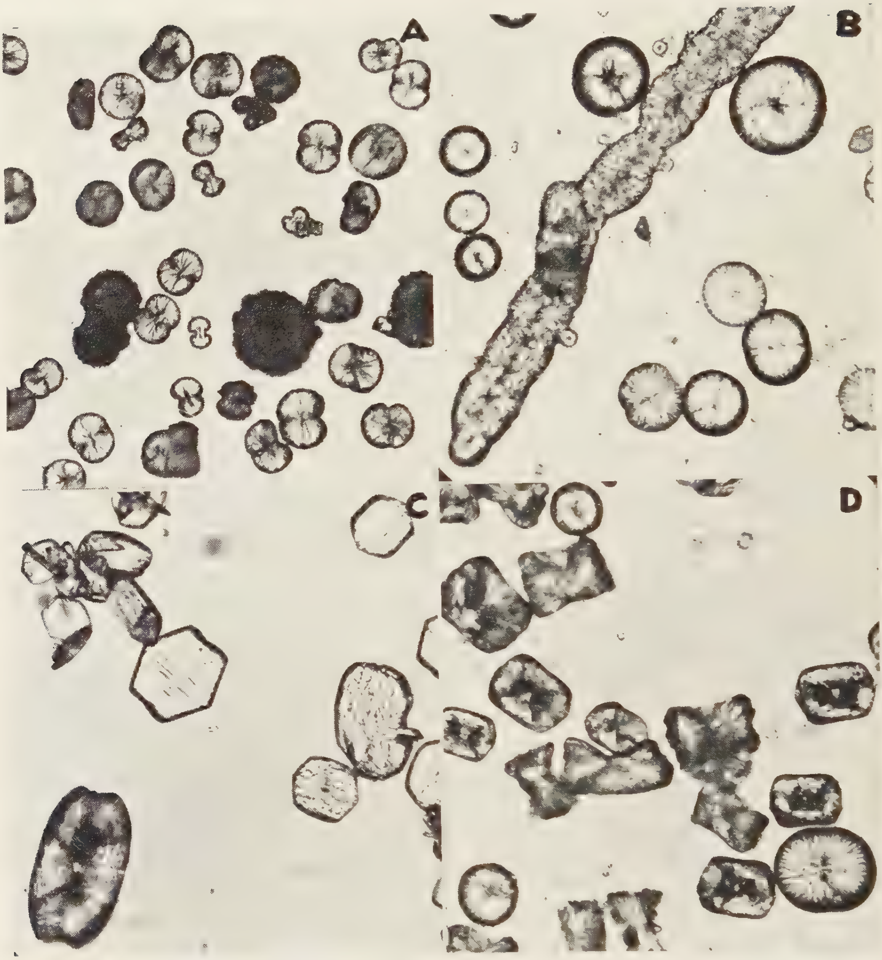

FIGURE 3. Urinary crystals of acetylsulfathiazole. (*A*) *Dumbbells* or "shocks of wheat with central binding": the crystals start as small slender sheaves and grow in such a way that the two half circle parts finally close to form a rosette (0.004 by 0.06 to 0.06 by 0.08 mm.). (*B*). *Rosettes* (0.04 to 0.1 mm.): the large rodlike structure, consisting of acetylsulfathiazole, may represent a "crystalline cast." On microscopic examination both crystal forms (*A* and *B*) appear amber green and show pronounced radial striation. (*C*) *Hexagonal platelets* (0.04 by 0.06 to 0.15 by 0.2 mm.): sometimes very thin, glass-clear, more often thick amber green with a distinct envelopelike pattern. Some of the crystals may reach considerable size, and two opposite edges may appear indented resulting in shapes as shown in the lower right corner of the photograph. (*D*) Simultaneous presence in the same urinary specimen of the three forms outlined in *A*, *B*, and *C*—a rather frequent observation. ×125. From Lehr & Antopol. 1942. Am. J. Clin. Pathol. **12:** 200.

and permanent damage or death;[22-24] it was likewise shown, however, that mixtures of partial dosages of these same compounds or single sulfonamides of high solubility, such as sulfanilamide, sulfacetamide, sulfadimetine, sulfisoxazole, and others, will have no deleterious effects upon the kidneys. Thus,

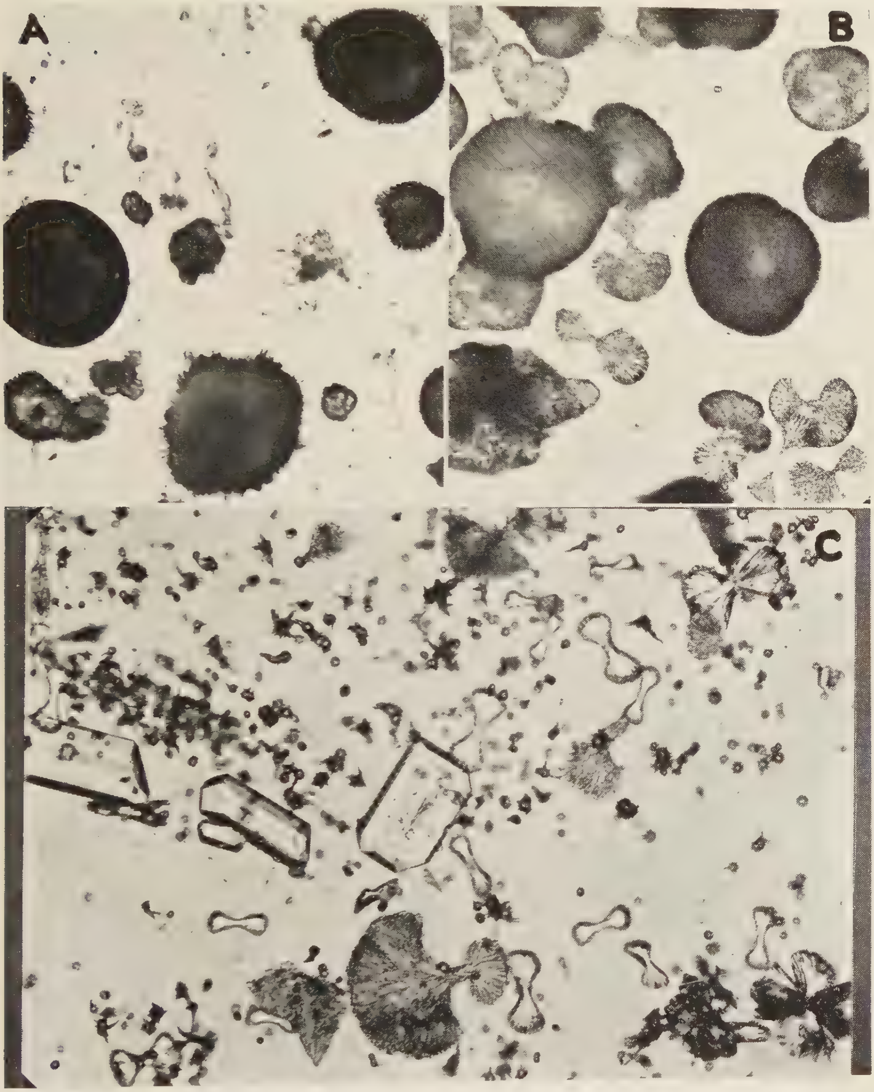

FIGURE 4. Urinary crystals of sulfadiazine and acetylsulfadiazine. (*A*) Dark, muddy green globules, sometimes covered with needlelike processes (chestnut burr forms), consisting almost entirely of free sulfadiazine (0.03 to 0.16 mm.). (*B*) Among globules containing free sulfadiazine, "shocks of wheat with eccentric binding" and "shell" forms, consisting mainly of acetylsulfadiazine. Like the corresponding sulfathiazole crystals, they are amber green and have a conspicuous radial striation (0.02 to 0.14 mm.). (*C*) Urinary sediment of a patient who had been switched from sulfathiazole to sulfadiazine treatment 24 hours before voiding the urine specimen containing these crystals. A perfect "shock of wheat with eccentric binding" (acetylsulfadiazine) can be seen among symmetrical dumbbells (acetylsulfathiazole). Also present are typical phosphate and monourate crystals. ×156. From Lehr & Antopol. 1942. Am J. Clin. Pathol. **12**: 200.

TABLE 3
SUBACUTE TOXICITY*
(Intraperitoneal Injection of Sodium Salts for 5 Consecutive Days in Albino Rats)

Drug		Dose (gm./kg.), partial	Dose (gm./kg.), total	No. of animals	Per cent death
Single	SD		0.9	25	100
	ST		0.9	15	100
	SMD		0.9	20	75
	SPZ		0.9	10	100
	SP		0.9	15	94
Mixture of two	SD	0.45	0.9	10	40
	ST	0.45			
	SD	0.45	0.9	10	40
	SMD	0.45			
Mixture of three	SD	0.3	0.9	10	0
	SMD	0.3			
	ST	0.3			
	SD	0.3	0.9	10	0
	SMD	0.3			
	SP	0.3			
	SD	0.3	0.9	10	0
	SMD	0.3			
	SPZ	0.3			

Symbols: sulfadiazine, SD; sulfathiazole, ST; sulfamerazine, SMD; sulfapyridine, SP; and sulfapyrazine, SPZ.
* From Lehr, D. 1947. Brit. Med. J. **2**: 943.

many years ago I demonstrated that sulfacetamide, given to albino rats by intraperitoneal injection in a dosage of 0.6 gm./kg. daily for a period of 9 weeks, did no harm whatsoever to the kidneys, although urine concentrations reached peak levels of about 6000 mg. per cent every day.[25] Similar experimental observations were made subsequently with sulfadimetine and sulfisoxazole.[26,27]

These facts lend strong support to a point of view I have held from the very beginning, namely, that it is primarily the physical factor of poor solubility, rather than a chemical nephrotoxic factor, that accounts for most renal damage from the sulfonamides.[23,25] The close relationship of sulfonamide solubility and renal complications is evident, not only in the animal experiment, but in human therapy as well (TABLE 4). The prevention of renal complications caused by sulfadiazine and its congeners by improving solubility relationships through adequate alkalization[28,29] or through employment of the sulfonamide mixtures[21,30,31] stresses again the primary importance of a purely mechanical factor.

Briefly the rationale for the use of sulfonamide mixtures is as follows (FIGURE 5): several sulfonamides, such as sulfathiazole, sulfadiazine, and sulfamerazine, can be dissolved simultaneously in the same medium almost to the full extent of their separate saturation levels without the occurrence of precipitation. Consequently, mixtures of partial dosages of several sulfonamides show substantially less tendency toward the development of renal obstruction by intra-

TABLE 4

RELATIONSHIP OF SOLUBILITY TO RENAL COMPLICATIONS*

Drug	Solubility in water, 37° C., *p*H 5.5, mg. %		Blockage of kidneys by crystalline deposits
	Free	N_4 Acetyl	
Sulfadiazine	13	20	Frequent
Sulfamerazine	37	79	Frequent
Sulfamethazine	75	115	Rare
Sulfapyrazine	5	5	Very frequent
Sulfathiazole	98	7	Very frequent
Sulfacetimide	1100	215	Not encountered
Sulfanilamide	1500	530	Not encountered

* From Lehr, D. 1950. Brit. Med. J. **2**: 601.

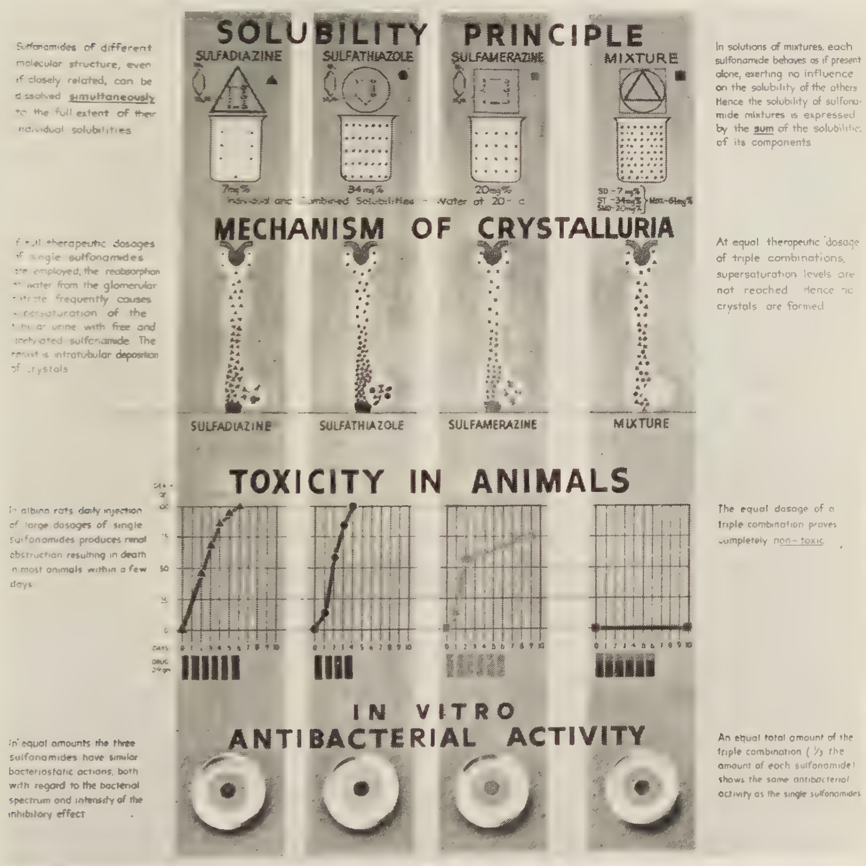

FIGURE 5. Experimental data.

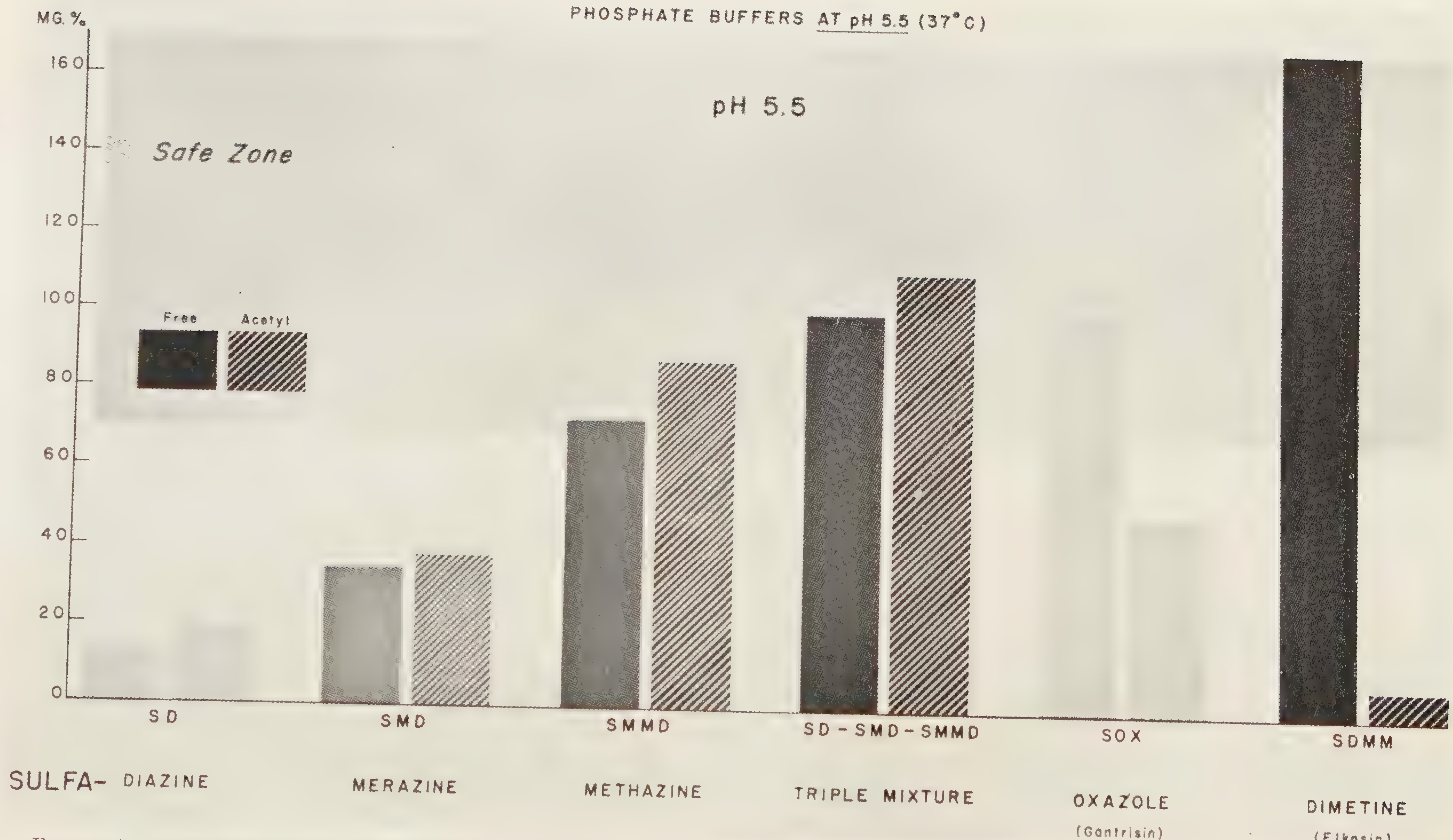

FIGURE 6. Solubilities of various sulfonamides. In tests with 1000 unselected patients from various disease groups it was found that more than 62 per cent of urine specimens have a *p*H of 5.5 or less (C. W. Eisele, General Hospital, Denver, Colo., unpublished data). The graph demonstrates that, in this acid *p*H range of human urine, only the triple sulfapyrimidine mixture, both in its free and acetylated form, as well as the *free* form of sulfadimetine and sulfisoxazole, possess solubilities that are safely above the danger zone with regard to the possibility of massive renal blockage.

tubular deposition of crystals than do any of the single components of the mixture in equal total dosage. This fact, proved by post-mortem examinations and chemical analyses of the blood, kidneys, and urine, explains the strikingly low toxicity of sulfonamide mixtures in the experimental animal. At identical total concentrations the *in vitro* antibacterial efficacy of sulfonamide mixtures is equal to the activity of its single constituents.

It stands to reason that if, for example, sulfathiazole, sulfadiazine, and sulfamerazine, when given separately in full therapeutic dosage, cause a high incidence of renal complications, and if this reaction can be practically eliminated by using the same dosage of a triple mixture (of equal partial amounts of these three compounds), the importance of a primary nephrotoxic factor appears negligible. In line with this evidence it should be remembered also that highly soluble sulfonamides that do not cause renal damage are otherwise as toxic as or often more toxic than sparingly soluble sulfonamides.

The existence of an independent chemical nephrotoxic factor has been postulated by many authors,[13, 32-36] especially on the basis of anuria occurring during sulfonamide therapy without apparent obstruction of the kidneys. Although experimental proof for a mechanism of this type is lacking, it could be argued that this reaction is specific to the human kidney. However, it should then have been observed as frequently with highly soluble as with sparingly soluble sulfonamides. Of course, the absence of crystalline deposits in the kidneys at autopsy, presented as evidence for the existence of a primary nephrotoxic factor, does not exclude the possibility of transient mechanical obstruction during life. Vilter and Blankenhorn[13] emphasized that "crystalline obstruction or 'calculi' may be asymptomatic." In this connection, it is of particular interest to note that anuria, reported to have occurred without apparent mechanical blockage, was almost invariably caused by sparingly soluble sulfonamides. I have found that if an experimental animal with transient renal obstruction from sulfonamide crystals is permitted to survive for a sufficient length of time, no trace of concretions may be left in the kidney at autopsy, although this organ may manifest a picture closely resembling that described as primary nephrotoxic damage in human beings.[37] On the other hand, there is little reason to doubt that, in mechanical obstruction, direct and extended contact of sulfonamide crystals with the tubular epithelium may result in secondary chemical irritation as well. Some human deaths in anuria due to "sulfonamide nephrosis," that is, without mechanical obstruction, might belong to the group of hypersensitivity reactions believed to entail parenchymatous degeneration and focal necrosis in organs other than the kidney.[38, 39]

When a battery of effective systemic sulfonamides became available at the bedside, the striking correlation between solubility and incidence of complications in the urinary tract, as depicted in TABLE 4, left no doubt concerning the overwhelming importance of mechanical renal blockage. It was therefore no accident that satisfactory solubility characteristics became the *conditio sine qua non* for the acceptability of a new systemic sulfonamide. This is apparent from the solubility characteristics of modern sulfonamide preparations (FIGURES 6 and 7).

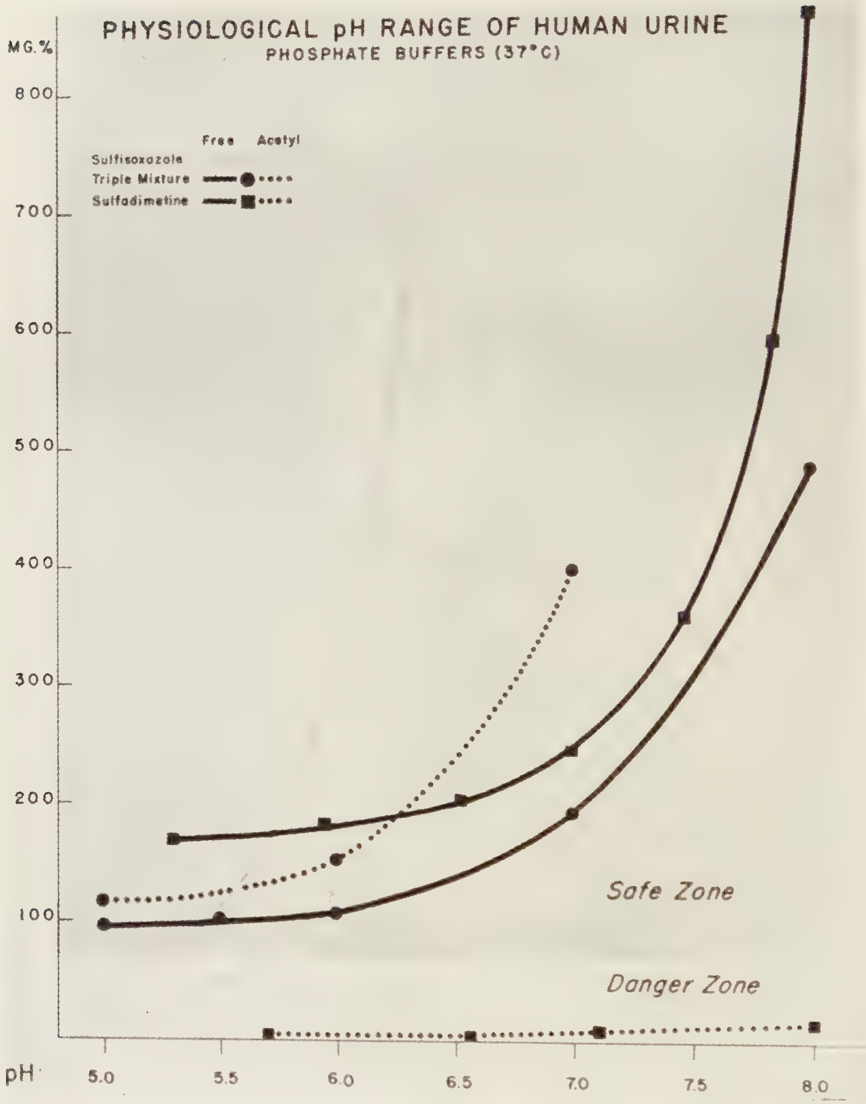

FIGURE 7. Solubilities of three modern sulfonamide preparations, determined over the entire physiological *p*H range of human urine. It is apparent that the solubility of acetyl sulfadimetine remains inadequate even in alkaline medium. Fortunately, the degree of acetylation of sulfadimetine is relatively low (10 to 30 per cent in human urine). Sulfisoxazole, on the other hand, despite the unsatisfactory solubility of its acetylated product at *p*H 5.5 and below, provides the greatest renal safety above a *p*H of 6. The sulfapyrimidine triple mixture and its acetylated product show satisfactory solubility over the entire *p*H range. (Based in part on data from A. R. Biamonte & G. H. Schneller. 1952. J. Am. Pharm. Assoc. Sci. Ed. **41**: 341.)

Thus, with the employment of modern sulfonamide preparations, serious renal complications, once dreaded because of their frequency and the grave danger they entailed, have become almost extinct today.

Sensitization Reactions

It should be realized from the start that clear-cut information cannot be expected on so ambiguous and confusing a manifestation of sulfonamide toxicity as sulfonamide allergy. Actually, sensitization reactions lack any strict standards of evaluation, giving wide leeway to conflicting opinions and interpretations. Drug fever and drug rash are considered the most common and the least disputable indicators of sensitization caused by the sulfonamides. The reservation must be made, however, that the truly allergic nature even of these reactions has been confirmed by subsequent testing only in exceptional instances. Without such tests, drug fever may be confused with a flare-up of the infection, and nonallergic skin reactions may be counted as sensitization.

Except for extremely rare exfoliative dermatitis, which may be fatal, drug fever and rash are not usually serious in themselves. It is contended, however, that if therapy is not stopped, there may emerge more ominous reactions such as dangerous blood dyscrasias, parenchymatous damage to the liver and kidneys, or hypersensitivity angitis—a disseminated necrotizing lesion of the arterial wall. In this connection, it should be emphasized that the allergic nature of these serious but fortunately rare manifestations of toxicity have not been fully established. In fact, especially with regard to blood dyscrasias, evidence has been presented in favor of direct and primary tissue toxicity of the sulfonamides[14] or a depletion of certain essential metabolites or vitamin factors as the responsible mechanism.[20, 40]

In view of this uncertainty and our inability to exclude the possibility of grave reactions, we are fully justified in insisting on prompt interruption of sulfonamide therapy at the first sign of sensitization and, in general, in withholding re-administration in the case of patients with a history of sulfonamide allergy for a minimum period of several years. Today, with the availability of a battery of powerful antibiotics, the physician is spared the tantalizing decision between depriving the patient abruptly, often at the height of a virulent infection, of the specific "antidote," or of risking dangerous reactions by continuation of chemotherapy—a problem faced not infrequently in the pioneer days of sulfonamide therapy.

Drug fever. Drug fever usually appears between the seventh to tenth day of sulfonamide therapy, but it may develop even after cessation of medication. It is often accompanied by chills, malaise, and rash. For the purpose of diagnosis, it is important to remember that a sudden febrile episode after fever and after all signs and symptoms of the infection have subsided, suggests drug fever, although good clinical judgment must exclude an exacerbation of the original infection. Usually the situation is not as simple as that, because the drug fever is superimposed upon the fever caused by the infection and may betray its presence only by a sudden rise that seems out of proportion to the clinical course of the disease. A dramatic drop of temperature within 24

hours after the offending drug is stopped and "fluids are forced" is almost pathognomonic of drug fever. In a large series of cases the relative frequency of fever due to sulfonamides was found to be: sulfadiazine, 1.55 per cent; sulfamerazine 3.98 per cent; sulfamethazine, 2.04 per cent;[41] and sulfisoxazole, 3 per cent.[42-44] According to the tabulation of Hawking and Lawrence,[45] the incidence for sulfadimetine is lower than that of sulfamerazine and sulfamethazine.

Drug rash (dermatitis medicamentosa). Skin eruptions from systemic sulfonamide therapy may be urticarial, morbilliform, scarlatiniform, erysiploid, petechial, purpuric, and pemphigoid. They seldom occur before the seventh day, except in previously sensitized individuals, and may appear as late as the third week. The rash is frequently accompanied by itching, malaise, and fever and usually spreads over the trunk, face, and extremities. The dermatitis may be mild and fleeting or severe and rather persistent. In rare instances alarming anaphylactic reactions may accompany its emergence. Since sensitization may last for several years, re-administration within that period may result in more serious reactions. Northey[16] reports the following percentages for drug rash from sulfadiazine sulfamerazine, and sulfamethazine, respectively: 1.49, 3.58, and 2.04 per cent. Sulfadimetine reactions lie in the range of its isomer sulfamethazine.[45] The incidence for sulfisoxazole was calculated at 1.96 per cent[41] on the basis of data derived from seven publications.[42, 43, 46-50]

Contact dermatitis (dermatitis venenata). The topical use of sulfonamides on skin and mucous membranes has fallen into disfavor because of a high incidence of allergic reactions. Skin eruptions are usually eczematous in character, limited at first to the immediate site of exposure, but often spreading to skin in other parts of the body. At first there is intradermal vesiculation, then rupture of the vesicles, with oozing, and finally the development of dried exudate.[51]

There is a paucity of exact statements concerning the incidence of sensitization after topical application. Many reports on the use of sulfonamide ointments in pyogenic dermatosis[52-56] do not mention the development of sensitization. On the other hand, Tate and Klorfajn[57] reported 55 cases of sulfonamide dermatitis among 2289 admissions to the skin department of a military hospital, and Dark[58] found 12 cases of dermatitis in 218 patients treated with sulfathiazole, an incidence of 5.5 per cent. Northey[16] estimates the average incidence of contact dermatitis from sulfathiazole as 2.2 per cent, and compares it with an incidence of 5 per cent dermatitis medicamentosa from the oral administration of this drug.

The wide variations in the incidence of contact dermatitis reported by various authors are probably due in part to the differences in the types of infection treated and the duration of therapy, but are undoubtedly also influenced by the condition of the skin. In many instances the skin is largely intact; hence absorption is at a minimum and sensitization does not occur. However, the eroded areas of eczema, or skin denuded of its protective outer layers by burns or wounds, will permit deep and extensive sulfonamide penetration. The result may be a high incidence of contact dermatitis.[59, 60] Systemic re-administration of a sulfonamide to patients sensitized locally

by previous topical application will often show exclusive or predominant reaction at the locally sensitized area.[58, 61-69]

Finally, the dependence of sensitization on absorbability and tissue concentration of sulfonamides can be inferred also from the excellent and exacting study of Sulzberger *et al.*[59] on human volunteers. These authors investigated the "incidence of dermatitis following treatment with measured amounts of different sulfonamides in the same vehicle, on standard lesions (third-degree burns), in men of the same age group, living in the same environment, on the same diets, at the same occupation, and under similar experimental conditions." The over-all incidence of dermatitis was found to be 19 per cent in 254 men. The distribution of sensitization for the four sulfonamides employed was 57 per cent for sodium sulfadiazine, 22 per cent for sulfanilamide, 7 per cent for sulfathiazole, and 5 per cent for sulfadiazine. Based on these remarkable results, the authors conclude that "the relative incidence of sensitization corresponded to the relative water solubility of the compounds the most soluble giving the highest incidence of sensitization."

It is interesting to note that the "worst offender," sulfathiazole, caused a relatively low incidence of reactions and that, by raising the solubility of sulfadiazine, this "least offender" could be made to induce contact dermatitis in more than 50 per cent of the subjects. This seems to me a most dramatic demonstration of the importance of sulfonamide tissue concentration in the development of allergic reactions.

Dosage and incidence of sensitization. The incidence of allergic reactions is largely dependent upon the nature of the allergenic substance and the amount and concentration of the antigen to which the individual is exposed. It is influenced by a great number of predisposing factors such as heredity, constitution, infection, immunization, and dysfunction of various organ systems, as well as meteorological and geographical conditions. Within limits, the frequency of allergic reactions will increase in direct proportion to the intensity of exposure to the allergen,[70-72] particularly in types of sensitization where the role of heredity is less marked. Apparently almost every individual can be sensitized by adequate contact.[73]

There should be little reason to doubt that this rule applies to all drugs that can act as full antigens or haptens, including the sulfonamides. Suggestions of a definite relation between dosage of sulfonamides and sensitization have been made repeatedly almost since the inception of their clinical application,[45, 71, 74, 75] and yet it is still widely believed that the incidence of allergic reactions from the sulfonamides is independent of the dosage used.[76]

In 1948 I challenged the view that there was no relationship between sulfonamide dosage and liability to sensitization reactions.[17] With the aid of figures obtained from the literature (TABLE 5), I showed that increasing sulfonamide dosage leads to greater frequency of sensitization reactions; for example, in the case of sulfathiazole, 2 to 4 gm. daily for 3 to 15 days produced no cases of rash and fever with this "worst offender" in 3584 patients; 6 gm. daily for 3 to 10 days produced 6.4 per cent of reactions in 2475 cases; and 6 to 10 gm. daily produced more than 10 per cent of such reactions. Similar differences were noted with increasing doses of sulfanilamide and sulfadiazine.

TABLE 5

RELATIONSHIP OF DOSAGE TO INCIDENCE OF SULFONAMIDE ALLERGY*

Drug	Daily dosage in grams	Days of treatment	No. of cases treated	Incidence of rash and fever
Sulfanilamide	1–2	120–240	188	1.0%
	1.5–4	7–21	1,687	0%
	6–9	5–10	1,407	>10.0%
Routine therapy†				7.2%
Sulfathiazole	2–4	3–15	3,584	0%
	6	3–10	2,475	>6.4%
	6–10	5–10	529	>10.0%
Routine therapy†				11.2%
Sulfadiazine	1	12–90	664,840	<0.5%
	2–4	3–20	18,185	0.07%
	4–6	7–14	500	1.8%
	6+	2–31	2,791	2.3%
Routine therapy†				2.9%

* From Lehr, D. 1948. Brit. Med. J. **2**: 543.
† These figures are based on a comprehensive compilation of the incidence of rash and fever reported in the literature.

On the basis of these observations, I suggested that sensitization was most unlikely to occur with dosage of less than 2 gm. of sulfonamide daily (blood levels of less than 5 mg. per cent). At that time I reported preliminary clinical experiences with mixtures of two or three sulfonamides (for example, sulfadiazine, sulfathiazole, and sulfamerazine), so that no more than 2 or 3 gm. of any individual drug were given daily. The results suggested that such mixtures not only reduced the danger to crystal formation in the urinary tract, but also diminished the sensitization liability (FIGURE 8).

However, the production of sulfonamide allergy should not be confused with the response of a sensitized individual in whom a single challenging dose, even if small, may elicit a vigorous and typical reaction. It might well be that this fact has contributed to the mistaken idea that the production of sensitization by sulfonamides may occur with small dosages as readily and as often as with full therapeutic amounts.

It is well known that patients sensitized to one sulfonamide do not necessarily always react to other members of this group;[77] some patients reacted to sulfadiazine, sulfathiazole, and sulfapyridine, but not the mother substance, sulfanilamide.[78] Others have shown sensitivity to all members of the sulfanilamide group and, in some instances, the sensitization has extended to other *p*-aminobenzene compounds such as sulfanilic acid, *p*-aminobenzoic acid, and procaine.[59, 79] In spite of this evidence of cross sensitization, it is generally agreed that the allergic reactions are more frequent in response to the original sensitizing drug; for example, Dowling *et al.*[80] observed reactions in 69 per cent of sensitized subjects when the original drug was re-administered, and in only 17 per cent when some other sulfonamide was given. In several patients

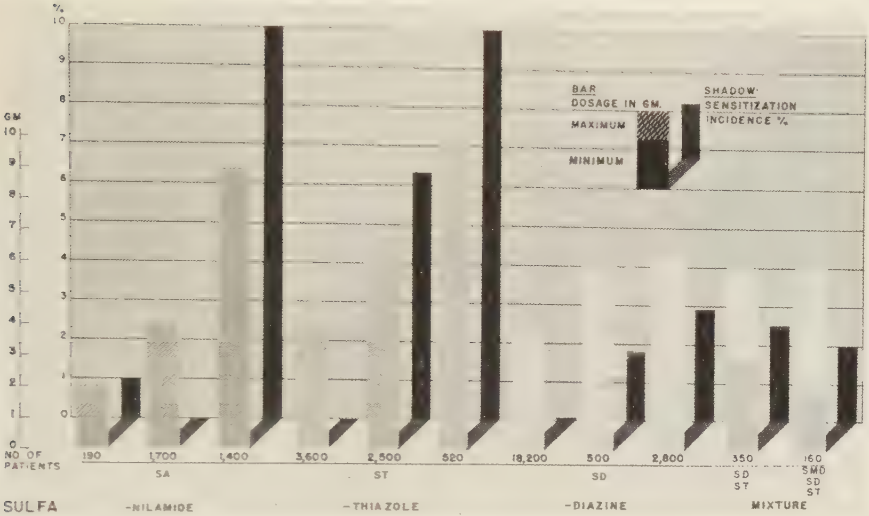

FIGURE 8. Dosage and incidence of sensitization reactions. The incidence of sensitization reactions increases in proportion to the *dosage* (tissue concentration) of single sulfonamides. Partial dosages of several sulfonamides (for example, double or triple mixtures) may cause dilution of tissue levels of individual sulfonamides beyond the minimum sensitizing concentration, provided that the components of the mixture are distinguished by the body as separate allergens. This would result in a *lowered* incidence of allergic reactions. If not distinguished as separate allergens, the mixed sulfonamides will act like the full routine dose of a single drug. Theoretically, therefore, only a *decreased* and no increased incidence of sensitization reactions should occur from the use of triple mixtures. Practically, this concept has been fully confirmed in ten years of experience at the bedside.

receiving high dosage of a sulfathiazole-sulfadiazine mixture, I observed development of sensitization to sulfathiazole, but not to sulfadiazine.[17] In the majority of patients the tissue cells were apparently able to distinguish even between closely related compounds with regard to their allergenic properties. In all such instances a mixture of several sulfonamides in partial dosages should make it possible to exert full therapeutic effects and yet to "dilute" individual drug concentrations in the tissues below the level at which sensitization reactions are apt to occur. It is obvious that under these conditions the simultaneous presence of several distinct potential allergens in "non-antigenic" concentrations should be of little consequence.

On the other hand, if the cells of the body do not distinguish between the various sulfonamides, the danger of sensitization should still not be greater than if the full therapeutic dosage of a single sulfonamide had been administered. Obviously, the highest possible incidence will be determined by that drug which, in a mixture of equal partial amounts, tends to induce allergic reactions most frequently. However, there should be no additive or even increased incidence of allergic reactions as long as individual sulfonamide concentrations are kept well below the level necessary for the production of sensitization to any one single sulfonamide in human beings with "normal" susceptibility. This concept seemed to be borne out by our continued ex-

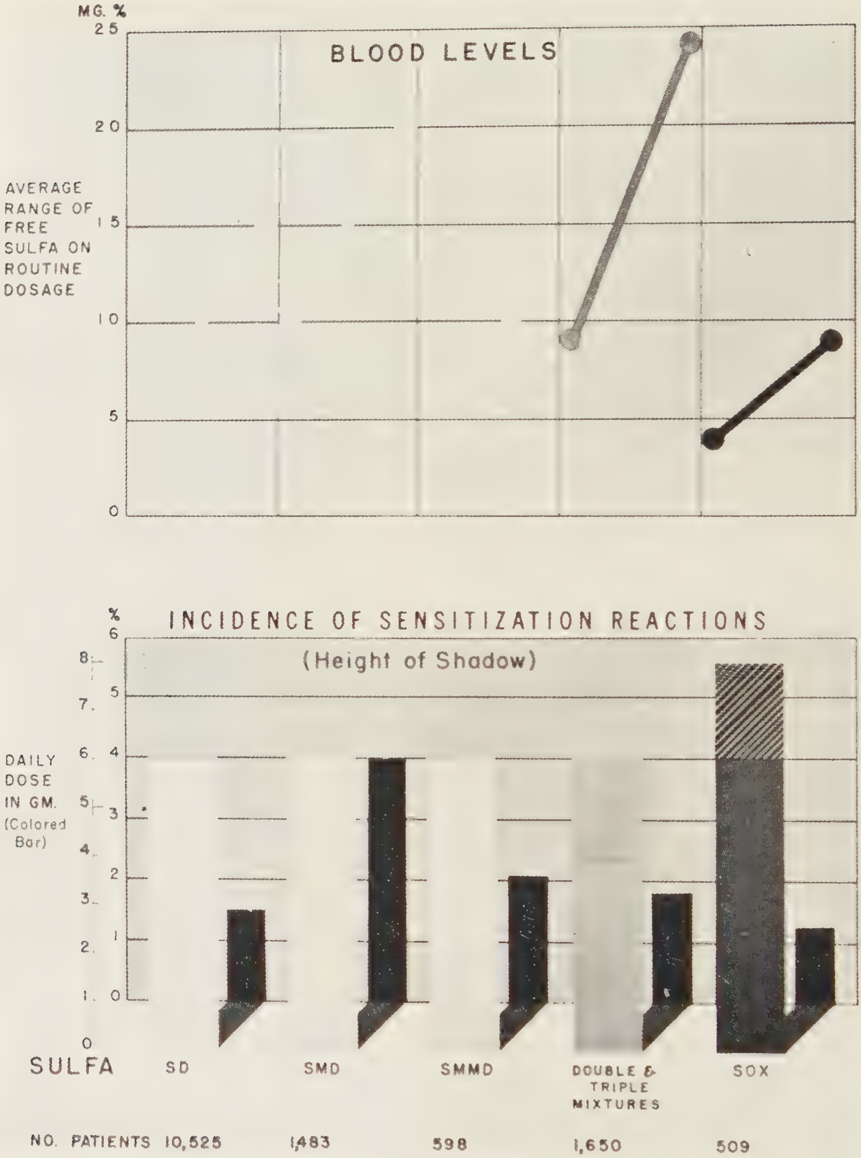

FIGURE 9. Blood levels and allergic reactions during routine therapy. Aside from the well-known differences in the specific allergenic properties of various sulfonamides, the incidence of allergic reactions observed in routine therapy appears to be related to the *height* of blood levels achieved with single sulfonamides. This applies apparently to sulfisoxazole as well as the three sulfapyrimidines, but not to sulfonamide mixtures. In fact, judging from mixture blood levels, such preparations manifest an incidence of sensitization that is usually lower than should be expected for any one single component of the mixture at the same high blood level.

perience with sulfonamide mixtures (FIGURE 9), and has not been challenged by the observations of other authors using this form of therapy.

Despite the extensive use of sulfonamide mixtures for a period of more than eleven years, sensitization reactions have remained in a surprisingly low range. This fact, more than any theoretical consideration, points towards the correctness of the concept.

Blood Dyscrasias

The more serious toxic effects upon the blood fortunately have always been of a low order of magnitude and have further declined in frequency since the introduction of sulfadiazine and its derivatives.

Mild leukopenia without clinically significant neutropenia occurs not infrequently and secondary anemia is rather common during prolonged sulfonamide therapy. Suppression of the bacterial flora of the gut and the consequent diminution in the production of vitamin factors are considered to be causative or contributory factors. Spontaneous and prompt recovery follows discontinuance of medication.

Agranulocytosis. This dreaded complication is a late reaction that occurs usually between the tenth and thirtieth day of full systemic therapy. With sulfadiazine the incidence is about 0.1 per cent.[81] It may appear suddenly or following increasing granulopenia. Therefore, white cell counts should be carried out whenever treatment is continued beyond the seventh day or earlier with the use of larger dosages, and should be repeated at frequent intervals (twice weekly) for the remainder of drug administration. If the granulocyte count falls below 2000 per cu. mm., therapy should be stopped at once, since the decline may continue for several days. With the disappearance of granulocytes from the blood stream there is increasing malaise, rise in temperature, sore throat, ulceration in the throat and mouth, enlarged cervical lymph glands, and final fatal septicemia.[82] The picture is not specific to the sulfonamides, but occurs with many other drugs. The mechanism is obscure, although sensitization has been suggested as one possibility, and direct toxic effect upon the bone marrow as the other. Treatment is purely symptomatic, consisting in the "forcing of fluids" and the use of antibiotics for control of the original infection and for protection against secondary invaders until the granulocyte count has returned to reasonable levels.

Much of the original dread has been removed by the knowledge that early discovery and antibiotic protection usually lead to recovery. This is the more reassuring since, even in the early days of sulfonamide therapy, before the introduction of antibiotics, proper precautionary measures have always kept fatalities to a minimum. Spink,[83] for instance, reported that no fatal case of agranulocytosis had developed among 2000 patients treated with sulfonamide compounds at the University of Minnesota hospitals, Minneapolis, Minn., and that every patient developing severe neutropenia had been given either sulfanilamide or sulfapyridine for more than two weeks.

Acute hemolytic anemia. This complication was seen most frequently with sulfanilamide, and is encountered only rarely with the sulfapyrimidines (0.05 per cent).[45, 81] Contrary to other toxic reactions, hemolytic anemia is seen

more frequently in children than in adults and is more prone to occur in the Negro. Sensitization to previous therapy is suspected in its causation, since the incidence of complication seems unrelated to dosage or blood concentration. The onset is precipitous some time between the third and eighth day of treatment, with fever, nausea, vertigo, leukocytosis, reticulocytosis, jaundice, rapidly increasing pallor, and shock. There is swelling of the liver and spleen, urobilinuria, and occasionally extensive hemoglobinuria, which may lead even to renal obstruction by hemoglobin casts. Blood hemoglobin values may drop to 30 per cent within 24 hours. With this fulminating form, mortality is rather high. Therapy consists in cessation of therapy, forcing of fluids and alkalis, and blood transfusions.

In one year Fox and Ottenberg[84] observed nine cases of acute hemolytic anemia due to sulfanilamide and sulfapyridine; six of these ended fatally. They reported finding three pigments in the blood serum: hemoglobin, methemoglobin, and Fairley's pigment methemalbumin. They pointed out that rapid destruction of erythrocytes and marked reduction in blood volume may be responsible in part for the clinical picture of shock and concluded that whole blood transfusions were of little benefit in therapy, possibly because of hemolysis of the new erythrocytes. They suggested the use of plasma instead.

After recovery such patients should never receive sulfonamides again, since recurrence of this complication is rather frequent. Mild hemolytic anemia is common after prolonged therapy and requires no particular precautions as long as hemoglobin values remain above 60 per cent.

Thrombocytopenic purpura and aplastic anemia. These reactions are extremely rare, and the theories for the possible mechanism of production are the same as those touched upon in the discussion of agranulocytosis. Immediate removal of the drug, antibiotic protection, blood transfusions or, in the case of aplastic anemia, transfusion of packed cells have been tried. The mortality from these complications is rather high.

Hepatitis

Hepatitis is very rare (less than 0.1 per cent with the newer sulfonamides). As in the case of the kidneys, a direct hepatotoxic effect and an allergic type of liver damage are assumed to occur. The complication may set in early during therapy, with nausea, vomiting, fever, enlargement and tenderness of the liver, jaundice, and decreased hepatic function. The jaundice must be differentiated from that occurring in hemolytic anemia or severe infections. Occasionally acute yellow atrophy develops. Hepatitis with diffuse necrosis has been described in exceptional instances, especially when accompanied by exfoliative dermatitis.[85, 86]

Pre-existing acute or chronic liver damage is no contraindication to the use of sulfonamides. Peterson *et al.*[87] failed to observe any aggravation during therapy. In fact, acute hepatitis in particular, if associated with bacterial infection, actually improved under sulfonamide therapy. However, severe toxic effects other than direct injury to the liver were unusually frequent in patients with portal cirrhosis.

Sulfonamide-induced hepatitis, on the other hand, requires immediate interruption of therapy, institution of procedures to hasten sulfonamide elimination from the body, and application of dietary and therapeutic measures commonly employed for hepatitis from any cause.

Milder Reactions

Acidosis

As already indicated, this reaction occurs only with compounds having a nonsubstituted sulfonamide group. Hence, it is specific for sulfanilamide and does not occur with any of the newer derivatives used at present in antibacterial therapy. However, recognition of the mechanism,[88] namely, inhibition of the enzyme carbonic anhydrase in the kidney and consequent excessive excretion of sodium, potassium, bicarbonate, and water by way of the urine, has led to the development of metabolic acidifiers derived from the sulfonamide series.[89] For the inhibition of carbonic anhydrase to occur, the $—SO_2NH_2$ group must be complete, although the *p*-amino group is not essential. Acetazoleamide (Diamox) and other similar compounds are presently employed as acidifiers and diuretics in many clinical conditions.

Cyanosis

Sulfanilamide is oxidized far more readily than any of its N_1-substituted derivatives. In fact, irradiation of an aqueous solution of this compound with ultraviolet light produces dark blue oxidation products within minutes. It is believed by some that these dark-colored oxidation products are responsible for the heliosensitivity of patients under sulfanilamide therapy, and are also the agents responsible for methemoglobin formation. This pigment would be expected from the analogy of the chemical structure of sulfanilamide to aniline, while the sulfamido group could give rise to sulfhemoglobin.

Cyanosis of the skin and mucous membranes was observed to a significant degree only after the administration of sulfanilamide. It was soon realized that this reaction was more frightening in appearance than in its objective effect upon the patient, since it did not interfere to any embarrassing degree with the oxygen-carrying power of the blood. Some authors felt that the color was due primarily to methemoglobin formation. Although other abnormal pigments were demonstrated (dark oxidation products of sulfanilamide), they occurred in quantities too small to contribute substantially to the discoloration.[90] The opposite view was taken by other investigators.[84, 91, 92] This difference of opinion has never been resolved. It has become purely academic, since cyanosis of any substantial degree is not encountered with any of the newer sulfonamides.

Central and Peripheral Nervous-System Reactions

An early review of this subject was presented by Little;[93] a more recent description was provided by Mackay.[94]

Mild nervous disturbances. Mild disturbances as a result of direct action of the sulfonamides upon the central nervous system are not infrequently encountered, although the newer sulfonamides show a markedly decreased incidence and degree if compared with sulfanilamide and sulfapyridine. The following reactions belong in this group: anorexia, nausea, vomiting, vertigo, tinnitus, lassitude, fatigue, ataxia, confusion, and mental depression.

Except for nausea and vomiting (where local factors such as gastric irritation may play a contributory role), which occasionally may still be troublesome, these signs and symptoms should cause no serious concern. They disappear readily and completely after discontinuation of the drug. Mild sedation is of help.

In ambulatory patients, some of these reactions encountered with sulfanilamide and sulfapyridine constituted a decided hazard, especially in occupations requiring constant attention and perfect muscular co-ordination, such as the operation of motor vehicles. Fortunately, controlled tests on normal men demonstrated no significant effect on reaction time, co-ordination, and mental efficiency during ordinary courses of sulfathiazole and sulfadiazine,[95, 96] so that this hazard appears remote at present.

Pyschosis. Psychotic manifestations in patients receiving sulfonamides are uncommon and, again, they are less apt to occur with sulfadiazine and its derivatives than with older sulfonamide compounds. The incidence appears to be influenced by the nature and severity of the infection, the pre-existing mental state of the patient, and the presence or absence of neurological disease. Severe infection, alcoholism, and previous "nervous breakdown" are believed to be predisposing factors. The psychosis is usually an acute delirium, with disorientation, overactivity and, often, visual and auditory hallucinations and paranoid or schizoid reactions.[94]

In 1825 consecutive patients with pneumonia treated with sulfadiazine by Stuart and Collen,[97] 14 (0.8 per cent) became psychotic. On the other hand, Johnstone and Fargacz[98] reported 5 psychotic reactions among 70 cases of meningococcal meningitis treated with sulfapyridine, an incidence of 7 per cent.

Peripheral neuritis. Apparently all systemic sulfonamides can produce this reaction,[99] although the now abandoned methylated compounds sulfanyl-dimethyl-sulfanilamide and sulfamethylthiazole seem to be more apt to induce this complication. The onset is sometimes delayed for weeks after sulfonamide administration has been discontinued.

According to Mackay[94] there is motor weakness, often without sensory disturbances, but tenderness, pain, hyperesthesias, and paraesthesias have been reported. Tendon reflexes are reduced or lost, while cutaneous reflexes are preserved. Recovery is slow and gradual but, as a rule, complete.

Over-all Incidence of Serious Toxic Reactions

The incidence of major toxic reactions was analyzed by Long[15] in 1947. The reactions included renal complications, rash, fever, acute hemolytic anemia, leukopenia, granulocytopenia, and liver damage—in other words, all those complications classified as serious earlier in this paper. The sum total of these side effects for four sulfonamide compounds was as follows: sulfanilamide,

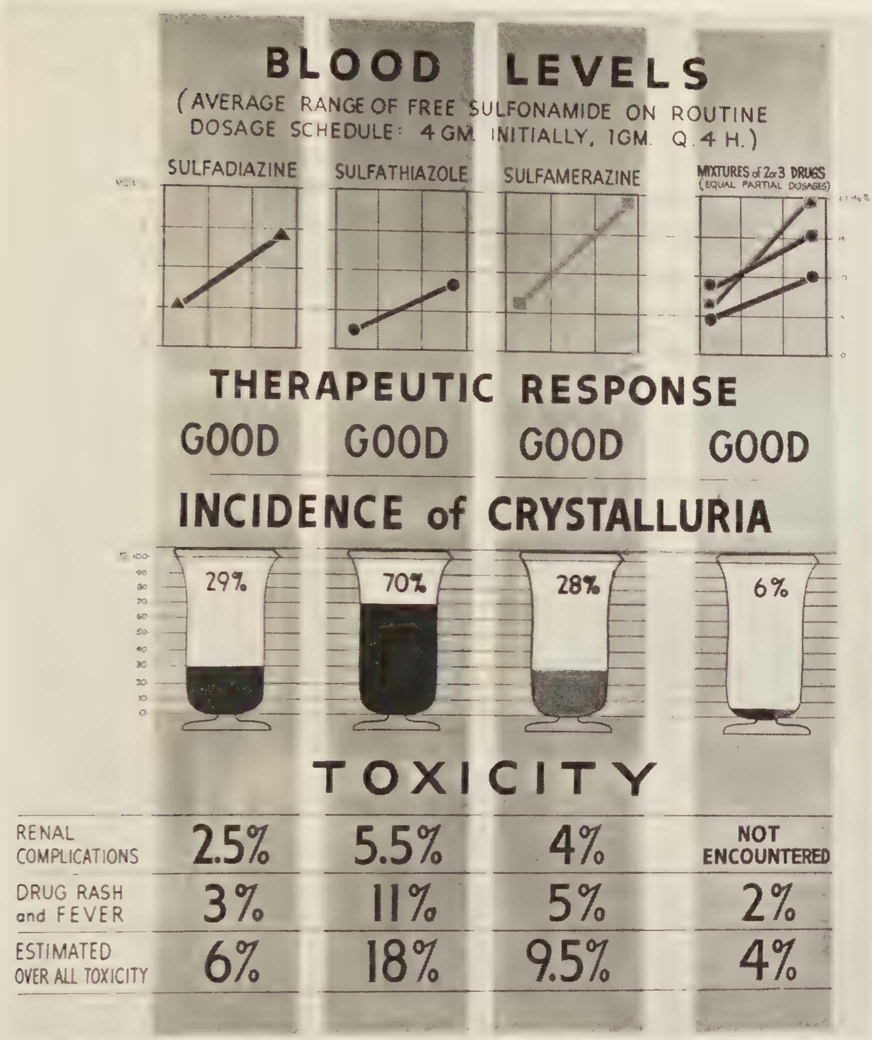

FIGURE 10. Clinical data compiled from the literature and from the author's own experience.

11.9 per cent; sulfapyridine, 15.9 per cent; sulfathiazole, 18.6 per cent; and sulfadiazine, 6.5 per cent.*

In 1948 I arrived at similar figures for sulfadiazine and sulfathiazole (6 and 18 per cent, respectively) on the basis of an independent compilation of data from the literature.[100] At that time I was able to add the value of 9.5 per cent for sulfamerazine (FIGURE 10). In this figure it can be seen that the higher

* These figures were based on the reported incidence of toxic reactions in a large series of clinical cases, amplified by Long's personal experience and, in the instance of sulfadiazine, on data furnished by M. Finland and his associates.

incidence for the monomethyl derivative of sulfadiazine is due primarily to an increase of both renal complications and sensitization reactions, and also that sulfonamide mixtures caused an over-all incidence of only 4 per cent, which was due to the elimination of renal complications and a low sensitization liability.

Comparable data on side effects induced by sulfamethazine, sulfadimetine, and sulfisoxazole are not as readily available. In the United States, sulfamethazine, while used extensively as a component of sulfonamide triple and quadruple mixtures, is employed only to a rather limited extent as a single drug for the treatment of systemic infections. This latter limitation applies also to its isomer, sulfadimetine. Although both compounds have undergone more substantial clinical evaluation in Great Britain[101, 102] and on the Continent,[103, 104] data on toxicity in large series of patients are not easily obtainable from the European literature. In a small series of patients with pneumonia, the incidence of side effects was reported as 7 per cent for sulfadiazine and 6 per cent for sulfamethazine.[101]

Sulfisoxazole, although used in recent years to a very large extent for the treatment of systemic infections has not yet been subjected to a final evaluation of its toxic potentialities. If one extracts, from the extensive tabulation of Kutscher *et al.*,[19] information on toxic reactions of sulfisoxazole comparable to that prepared by Long,[15] one arrives at a minimum possible incidence of 2.8 per cent (the total number of recipients of the drug who manifested the specific reactions) and a maximum possible incidence of 8.8 per cent (the percentage of patients studied who manifested the specific reactions). The evaluation is based on a total of 2010 patients receiving sulfisoxazole. The true incidence lies perhaps somewhere between these two figures. The data on sulfadimetine contained in Kutscher's tabulation are inadequate for analysis, but they suggest, at least, a very low incidence of toxic reactions for this compound.

On the basis of information available at this time, it would seem that sulfamethazine and probably also sulfisoxazole, despite their low potential for the production of renal complication, do not surpass to any substantial degree the excellent toxicity record of sulfadiazine. In this connection it should be remembered that the dimethylated sulfapyrimidines and sulfisoxazole are characterized by blood maintenance levels considerably below those of sulfadiazine, and that, within limits, the incidence of all toxic complications is directly proportional to the height of the blood and tissue levels of a sulfonamide. This was exemplified for sulfadiazine in a detailed analysis of reactions occurring during treatment of 1357 patients by Plummer and Wheeler.[11] This showed the following relationship between blood concentrations and toxic reactions: at blood levels of less than 5 mg. per cent sulfadiazine there were 1.8 per cent toxic reactions; at 5 to 10 mg. per cent the figure rose to 7 per cent; at 10 to 15 mg. per cent it increased to 12.8 per cent; and at more than 15 mg. per cent it reached 14.8 per cent. It is clear, therefore that, at equal dosage levels, sulfonamides of equal toxicity that give lower maintenance levels in the blood should show a correspondingly lower incidence of toxic complications.

In the light of this reasoning, the high figures for reactions from sulfanilamide

and sulfathiazole serve to emphasize the considerable toxicity of these compounds, since the maintenance levels of both are of a low order of magnitude, and sulfanilamide does not cause mechanical obstruction in the urinary tract. Similarly, in comparing the clinical toxicity of sulfadiazine with that of sulfamethazine, sulfadimetine, and sulfisoxazole on a dosage basis, one should remember that the lower maintenance blood levels of the three dimethylated sulfonamides may make them appear less toxic than they actually are.

Summary

Re-evaluation of the clinical toxicity of the sulfonamides after two decades of their employment as antimicrobial agents indicates that continued research and development in this field of chemotherapy has resulted in a dramatic reduction of the incidence of all toxic reactions.

The introduction of sulfadiazine and, in particular, its homologues has minimized nausea, vomiting, and other central nervous system side effects. It has also substantially reduced the incidence of sensitization reactions and of blood dyscrasias. Finally, it has made the dreaded mechanical obstruction of the kidneys amenable to control by alkalization. With the use of the sulfonamide-mixture principle, almost complete renal safety has been added to these advantages of the sulfapyrimidines. With the development of highly soluble heterocyclic derivatives of sulfanilamide, reliable protection of the kidneys has also been achieved.

Thus, modern sulfonamide therapy has reached a remarkable degree of safety, insuring maximum therapeutic efficacy at a minimum risk to the patient, especially with regard to renal complications and sensitization reactions. This should not create a false sense of security, however, and should not detract from the fact that the sulfonamides remain potentially dangerous agents. Their clinical application requires caution and alertness on the part of the physician in order to forestall serious complications. Avoidance of prolonged, intensive sulfonamide therapy, whenever feasible, and prompt interruption of drug administration when potentially dangerous reactions emerge, will aid substantially in the achievement of this goal.

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EXPERIMENTAL ACTIVITIES OF NEW SULFONAMIDES

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In 1942, Bell and Roblin[1] stated that the more negative the $—SO_2$ group of an N_1-substituted sulfonamide derivative, the greater its bacteriostatic power. According to Northey,[2] this would indicate that, if the action of these compounds is dependent upon the inhibition of enzyme systems involving *para*-aminobenzoic acid (PABA), it is unlikely that new sulfonamide derivatives will be found that are appreciably more active *in vitro* than sulfathiazole and sulfadiazine. The therapeutic fitness of a chemotherapeutic remedy, however, by no means depends exclusively upon its intrinsic potency as expressed by *in vitro* activity. Many secondary factors exert a decisive influence. Although high toxicity and the occurrence of untoward side effects may eliminate a compound highly active intrinsically, an excellent tolerance, a strong penetration into the organs in which high concentrations are therapeutically desirable, or a low incidence of over-all side effects may favor compounds of lower intrinsic activity. Indeed, since the introduction of sulfadiazine, a number of other sulfonamides that are much less active *in vitro* than sulfathiazole or sulfadiazine have appeared; nevertheless, they have proved to be excellent chemotherapeutic agents, with certain advantages over other sulfonamide derivatives.

Therapeutic interest often shifts from established indications to other previously neglected considerations. In the early years sulfonamides were used mainly in the treatment of acute general infectious diseases. Subsequently, however, remedies were found, mainly antibiotics, that were superior to sulfonamides in this field. Interest in sulfonamides then changed to specific therapeutic indications in which the more powerful antibiotics did not give satisfactory results, or in which their application was connected with certain risks or unpleasant side effects. Chronic infectious diseases often turned out to be resistant to various forms of antibiotic treatment, whereas the sulfonamides maintained their place in this field of chemotherapy. Urinary infections, for instance, often resisted all antibiotics, whereas sulfonamides were discovered that were rapidly absorbed after peroral administration and were eliminated in high concentrations by the kidney, thus proving most valuable for treating this type of infection. On the other hand, other sulfonamides came into prominence because they were practically not absorbed at all by the intestinal tract; and they were thus able to exert local activity in various intestinal infections. Lately interest has increased in derivatives that are well absorbed but rather slowly eliminated, so that they remain in the body tissues for a prolonged period, providing a sufficient therapeutic effect with only one or two doses within twenty-four hours. Indeed, even in a field so well explored as that of the sulfonamides, therapeutic surprises are still possible.

New indications for sulfonamides have been found; for instance, their use in histoplasmosis (Mayer *et al.*[3]), in South American blastomycosis, and now in diabetes mellitus. The search continues for new derivatives with specific properties or for derivatives with new activities. The characterization of a sulfa drug's activity against infectious diseases is still based on the following factors: (1) the dose necessary to provide a curative effect; (2) the duration of the effect; (3) the blood concentration necessary for the curative effect; (4) the excretion in the urine; and (5) the tolerance (toxicity).

The *dose* necessary for a curative effect depends chiefly on the antibacterial activity of the compound, but this can be modified by factors such as absorption and excretion. Among compounds that have a similar qualitative effect, the most effective will be the one that produces a curative effect in the lowest doses.

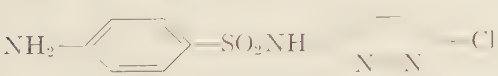
The *duration of the effect* depends largely on the length of time a compound remains in the organism in its active form. With substances otherwise equal in effect, we must expect that a compound with a long duration of effect either will be degraded or excreted more slowly.

The *blood concentration* represents an average value for the concentration of a substance necessary to achieve the curative effect, without regard to the concentrations in individual organs. The more effective a preparation is and the lower the doses necessary to obtain a curative effect, the lower will be the blood concentration. Likewise, the duration of the drug's influence must be considered, since the prolonged activity of a low concentration can produce the same result as a short-lived effect of a higher concentration.

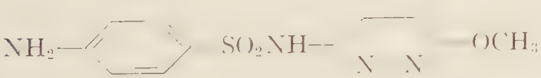
In general, *excretion* in the urine will parallel the blood concentration. High blood concentrations are usually followed by a correspondingly high excretion, unless special conditions impair the renal excretion. The rapidly excreted substances yielding high urinary concentrations are especially suitable for treating infections of the urinary tract, while the slowly excreted compounds are less suitable for this purpose.

A new sulfonamide will show clinical promise if it has distinct advantages compared with the familiar preparations in regard to one or several of these properties, and if its toxicity and tolerance are favorable.

The following experiments deal with comparisons of four sulfonamides that correspond in different ways to these various factors. Two well-known derivatives, sulfadiazine and sulfisoxazole, have been compared with two new compounds, the 3-sulfanilamido-6-chlor-pyridazine and the corresponding 6-methoxy derivative.



sulfachloropyridazine (Ba-10370)



sulfamethoxypyridazine (Ba-17429)

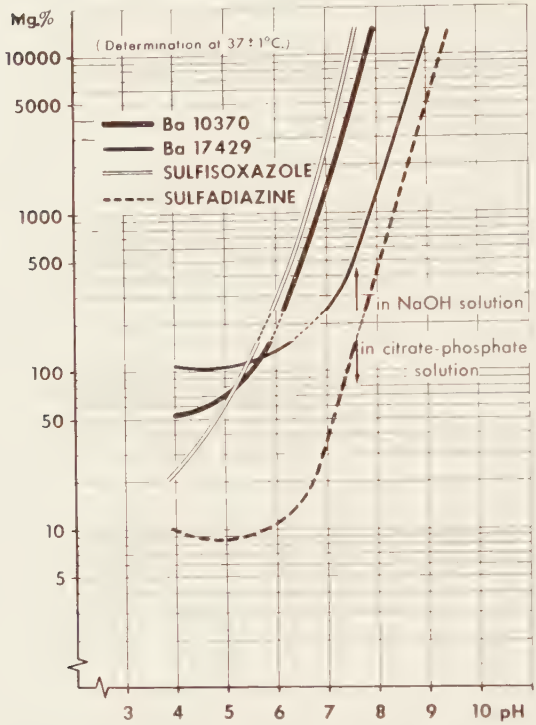


FIGURE 1. Solubilities of sulfachloropyridazine (Ba-10370), sulfamethoxypyridazine (Ba-17429), sulfisoxazole, and sulfadiazine.

The solubility of some of these compounds differs widely. In a buffered medium and at a temperature of 37° C., sulfachloropyridazine and sulfisoxazole show the best solubility in a *p*H range of 6.5 to 7; their solubility is at least 25 times better than that of sulfadiazine, and 10 times better than that of sulfamethoxypyridazine (FIGURE 1).

From these data it is to be expected that sulfachloropyridazine, Ba-10370, like sulfisoxazole, will be more suitable for the treatment of urinary infections, whereas the corresponding methoxy derivative, Ba-17429, the less soluble compound, will be more slowly eliminated and will not provide comparably high urinary concentrations.

Chemotherapeutic Effect

For characterization of the chemotherapeutic effect, we tested the preparations in the usual manner, not only against a streptococcal sepsis in mice, but also against localized infections of the urinary tract and as intestinal disinfectants. Groups of at least 10 mice were infected with about 1000 times the minimal lethal dose of streptococci and were treated for 1 or 6 days—in the latter case once daily—perorally with different doses of sulfachloropyridazine and the comparative sulfonamides. After 10 days the percentage of

TABLE 1

CHEMOTHERAPEUTIC EFFECT

Infection with:	Daily dose during 6 days, mg., kg., P.O.	Percentage survival after 10 days			
		Sulfa-chloro-pyridazine	Sulfa-methoxy-pyridazine	Sulfi-soxazole	Sulfa-diazine
Strep. hemolyticus 38	50	80	80	30	100
	100	87	80	50	100
Strep. hemolyticus 10	50	87	—	50	—
	100	100	—	95	83
Past. avicida 341	50	100	—	100	100
E. coli 205	50	80	67	50	67
Ps. pyocyanea 313	250	75	67	50	100

cured animals was determined (at this time all controls had died). In the experiment reproduced in TABLE 1, for example, the curative effects in percentages were observed in infections with *Streptococcus hemolyticus 38* and other bacteria.

The effect was improved for the readily soluble substances (sulfachloropyridazine and sulfisoxazole) by subdividing the daily dose into several small doses; but this improvement was not possible for sulfadiazine. Thus, quantitatively and qualitatively, the spectrum of activity of sulfachloropyridazine was very similar to that of sulfadiazine and sulfamethoxypyridazine; their chemotherapeutic effect against streptococci and *Escherichia coli* was pronounced.

Experiments on Urinary Disinfection

These experiments were carried out by two methods:

In vivo-vitro test. Doses of 250 and 100 mg./kg. were given perorally to rats. After 2 hr. the urine was withdrawn aseptically and the bacteriostatic effect was tested; the urine was diluted with glucose broth, infected with *E. coli*, and incubated for 24 hr. at 37° C. The dilution at which minimal bacterial growth occurred was taken as the bacteriostatic level. The bactericidal effect could be determined semiquantitatively by inoculating a given volume of these different urine dilutions on Leifson agar plates and counting the number of colonies. The results are presented in TABLES 2 AND 3.

This *in vivo-vitro* test, which demonstrates the rapidity with which the substance was excreted in the urine and the intensity of the sulfonamide effect, was supplemented by the following test on the *in vivo* effect.

In vivo experiments. Groups of 3 to 6 rats, each with abundant urinary flora, were given the different sulfonamides, 250 mg. kg. and 10 mg. kg. daily by mouth in 2 divided doses for 7 or 14 days. The activity of the preparations against the *E. coli* flora was tested before and during treatment 3 times a week, that is, at 2-day intervals, by inoculating the urine on Leifson agar plates and

TABLE 2
URINARY DISINFECTION

	Controls	100 mg./kg.			
		Sulfa-chloro-pyridazine	Sulfa-methoxy-pyridazine	Sulfi-soxazole	Sulfa-diazine
Dilution of urine still active bacteriostatically	No inhibition 1:1	1:3200	1:100	1:800	1:10
Number of colonies on Leifson agar plates (growth after inoculation of this dilution)	Unlimited growth	2400	10,800	12,000	12,000

Sulfachloropyridazine was excreted in the urine in more effective concentrations than the other compounds. Thus, here we find a confirmation of the good disinfecting effect shown by sulfachloropyridazine *in vitro*, especially when tested in urine on *E. coli* (TABLE 3).

TABLE 3
BACTERIOSTATIC ACTIVITY OF SULFACHLOROPYRIDAZINE, SULFISOXAZOLE, AND SULFADIAZINE AGAINST *E. COLI* 5×10^{-6} (*p*H 6.2)

	Sulfachloropyridazine	Sulfisoxazole	Sulfadiazine
1: 3,200	0*	+	+
1: 6,400	0	+	+
1: 12,800	0	+	+
1: 25,600	0	+	+
1: 51,200	0	+	+
1: 102,400	+	+	+
1: 204,800	+	+	+

* Symbols; 0 denotes inhibition of growth; + denotes growth.

TABLE 4
URINARY DISINFECTION *IN VIVO*

Time	Survival of viable organisms in the urine			
	Sulfachloro-pyridazine	Sulfa-methoxy-pyridazine	Sulfi-soxazole	Sulfadiazine
	250 mg./kg.			
First week of treatment	0%	34%	6%	31%
Second week of treatment	0%	2%	18%	39%
	10 mg./kg.			
First week of treatment	9%	91%	30%	no reduction
Second week of treatment	5%	71%	15%	

Dose = 250 mg./kg., P. O. (divided into 2 doses) daily for 14 days; test of activity = counts of the surviving *E. coli* in the urine, diluted 1:100 on Leifson agar plates (average values for 6 rats).

TABLE 5
INTESTINAL DISINFECTION

Time	Survival of viable organisms in the intestine			
	Sulfachloro-pyridazine	Sulfamethoxy-pyridazine	Sulfisoxazole	Sulfadiazine
First week of treatment	8%	0.3%	50%	5%
Second week of treatment	4%	0.5%	43%	3%

counting the red *E. coli* colonies after incubation at 44° C. for 18 hr. These results are given in TABLE 4.

The urine-disinfecting action of sulfachloropyridazine was confirmed in numerous experiments. This action of sulfachloropyridazine may be attributed not only to its strong inhibiting effect on *E. coli*, but also to a rapid excretion, due to its solubility, in relatively high concentration. In regard to urine-disinfecting action, sulfachloropyridazine was superior to the substances examined.

With doses lower than 10.0 mg. kg., the superiority of sulfachloropyridazine was even more distinct, even in comparison to all other active sulfonamides.

Intestinal Disinfection

The good effect against *E. coli* organisms was apparent also with respect to intestinal-disinfecting action. In spite of its good solubility and absorption, sulfachloropyridazine reduced the number of *E. coli* present in the intestines, so that we may assume that the concentrations necessary for this purpose were lower than with other sulfonamides (TABLE 5).

Blood Concentration and Excretion

The blood levels and excretion were examined in dogs (FIGURES 2 AND 3). With all substances following peroral administration of 100 mg. kg., we found the maximal blood concentrations between 1 and 3 hr. after administration. The concentrations of sulfachloropyridazine and sulfisoxazole fell within 24 hr. to the initial values, whereas those of the methoxy derivative remained increased for more than 48 hr. The excretion in the urine corresponded to this behavior—that is, was maximal after 3 and 6 hr. for sulfachloropyridazine and sulfisoxazole—while sulfamethoxypyridazine was excreted in large quantities only after 1½ days, without attaining values similar to those obtained with the chloro derivative. Altogether, 100 per cent of the dose of sulfachloropyridazine and sulfisoxazole administered was excreted in the urine, whereas the corresponding quantities for the methoxy derivative and sulfadiazine were only 49 and 51 per cent, respectively. The clinical experience parallels this behavior, indicating that sulfachloropyridazine and sulfisoxazole are particularly useful in the treatment of infections of the urinary tract.

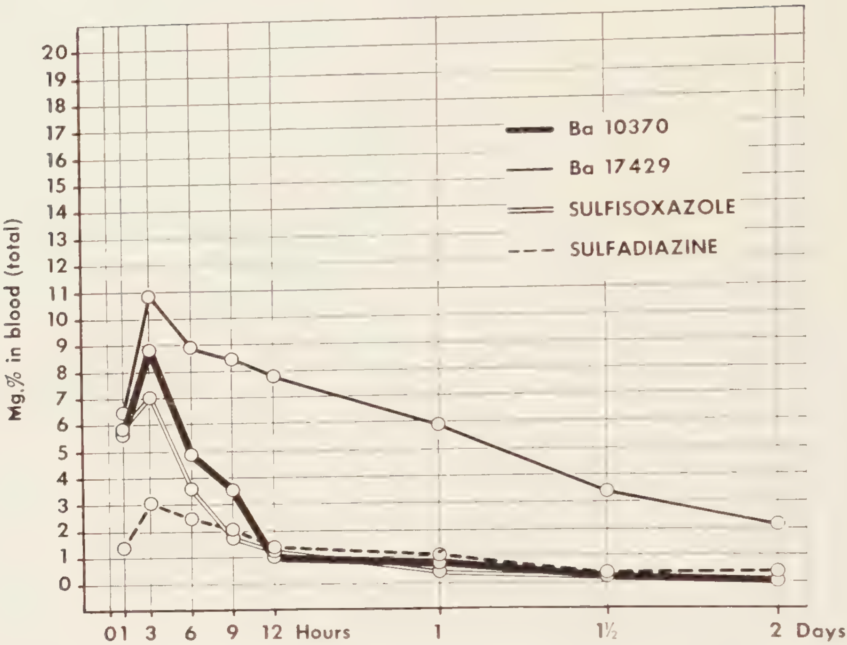


FIGURE 2. Blood concentration levels in the dog after 0.100 gm./kg. P. O. (2 animals).

Toxicity

The results of acute toxicity tests in mice following intravenous administration (in the form of the sodium salt) and peroral administration are shown in TABLE 6. It is evident from these data that sulfachloropyridazine, perorally administered, had the greatest toxicity of the compounds examined, while sulfadiazine was the least toxic. We may assume that compounds that produce a high concentration in the organism within a short time have a greater toxicity on peroral administration than substances more slowly absorbed. The sulfonamides effective in urinary infections and rapidly absorbed and more rapidly excreted in higher concentrations did not appear among the substances least toxic on peroral administration; in general, because of their physical-chemical properties, they displayed a higher toxicity than more slowly absorbed derivatives. This was not too important from the practical point of view, however, if no side effects due to toxicity appeared after chronic administration of therapeutically active doses. Clinical experience has confirmed this, inasmuch as no toxic phenomena have been observed thus far in more than 1000 patients treated with sulfachloropyridazine; whereas sulfadiazine, which is the least toxic in mice, can produce severe renal damage.

The comparison of these two sulfonamides, so closely related chemically, revealed numerous biological differences. The chloro compound was eliminated rapidly from the blood stream and led quickly to high levels in the urine;

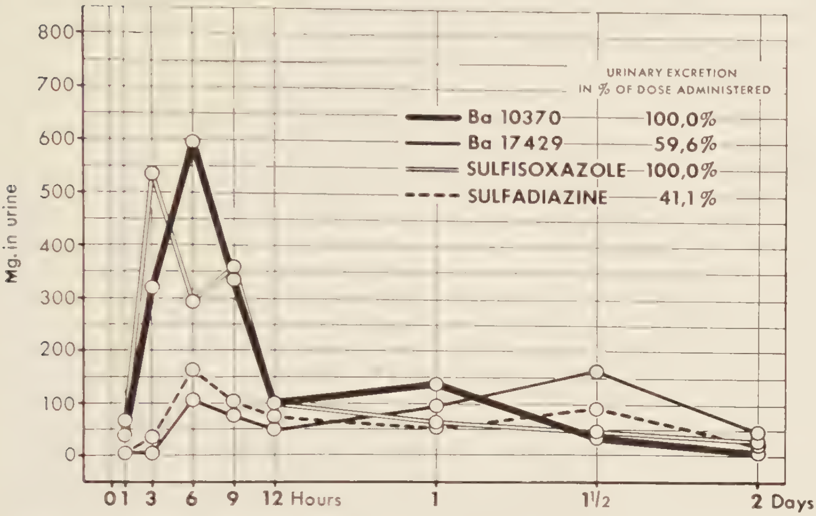


FIGURE 3. Urinary excretion levels in the dog after 0.100 gm./kg. P. O. (2 animals).

TABLE 6
TOXICITY

	Acute toxicity LD_{50} mg./kg.			
	Sulfachloropyridazine	Sulfamethoxypyridazine	Sulfisoxazole	Sulfadiazine
Mouse, I. V.	1,100	900	2,700	540
Mouse, P. O.	2,000	10,000	6,000	29,000

on the other hand, the methoxy derivative remained longer in the blood stream and, consequently, was eliminated more slowly. These differences illustrate again the fact—so unfortunate for the student of new chemotherapeutic compounds—that physiochemical and biological properties are unpredictable. As in many other instances, a very slight modification of the molecule produces a considerable biological variation.

The findings reported here indicate that the two differently substituted sulfonamidopyridazine derivatives are among the most active sulfonamides known. The fact that slight chemical modifications produce such sharply differing chemotherapeutic properties suggests that the two substances differ in the type of indication for which they can be used therapeutically. The high activity of sulfachloropyridazine against *E. coli*, its rapid and highly concentrated urinary excretion, and the fact that it is clinically well tolerated, render this preparation especially suitable for the treatment of urinary tract

infections, a category of infection in which animal experiments have shown it to be superior to other sulfonamides.

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EXPERIMENTAL INVESTIGATIONS OF SULFAMETHOXYPYRIDAZINE

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Of a series of sulfanilamidopyridazines synthesized by Clark *et al.*,[1] Kynex† sulfamethoxypyridazine (3-sulfanilamido-6-methoxypyridazine) appeared to be of particular interest because of a combination of adequate solubility and antibacterial activity combined with a low rate of urinary excretion. The structure of sulfamethoxypyridazine (SMP) is shown in FIGURE 1. The solubilities of the compound and its N[4]-acetyl derivative are listed in TABLE 1, with similar data for sulfadiazine and sulfisoxazole. Standardized bacterial infections in mice, as described by White *et al.*,[3] were used by Kuck[4] to compare the antibacterial potency of SMP with that of sulfadiazine. In the treatment of pneumococcal, streptococcal, and *Pasteurella* infections, Kuck found no significant difference in the potency of these two drugs on a basis of either blood level or oral dosage. In the *Klebsiella* infection, SMP was about half as potent as sulfadiazine.

This report is concerned primarily with studies on SMP with respect to its absorption, distribution, and excretion in dogs; conjugation in rats; acute toxicity in mice and dogs; and chronic toxicity in rats and dogs.

General Materials and Methods

The dogs used in these studies were beagles bred and raised in our laboratory and kept in groups in runs. The mice were of strain CF No. 1 and the rats were of modified Wistar strain; both were supplied by Carworth Farms, New City, N. Y. All animals were housed in air conditioned rooms (average temperature 74° F., range 68° to 79° F.) under artificial light (12 hours light, 12 hours dark). Dogs were fed once a day with a mixture of raw horse meat and Ken-L-Meal‡ in approximately equal proportions by weight. Water was given *ad libitum*. Rats were fed finely ground Purina Dog Chow Kibbled Meal and water *ad libitum*. Concentrations of SMP or other sulfonamides were determined by the method of Bratton and Marshall.[5] For determination of total sulfonamides (free plus conjugated§), hydrolysis was carried out with 0.2 N HCl for 1 hr. in a boiling water bath. Standard laboratory procedures[6] were used for analyses on blood and urine.

For post-mortem studies, dogs were sacrificed by intravenous injection of pentobarbital, rats by chloroform inhalation. In both species, special attention was given to the occurrence of concretions in kidneys, ureters, and bladder. The renal pelves, as well as the renal substances, were examined under the dissection microscope. Kidneys and livers were weighed in all autopsies;

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§ By conjugated sulfonamide is meant a derivative, presumably N[4] acetylsulfonamide, which reacts to give a color in the Bratton-Marshall method only after hydrolysis.

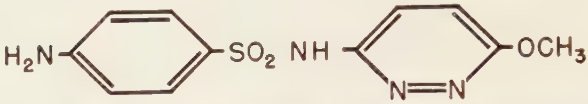

FIGURE 1. Kynex® sulfamethoxypyridazine (3-sulfanilamido-6-methoxypyridazine).

TABLE 1
SOLUBILITIES OF CERTAIN SULFONAMIDES

	Solubility at 37° C., mg./100 ml.*						
	Sulfonamide				N^4-acetylsulfonamide		
	*p*H						
	5	6	6.5	7	5	6	7
Sulfamethoxypyridazine[1]	110	120	147	—	35	40	75
Sulfadiazine[2]	10	18	—	60	16	36	222
Sulfisoxazole[2]	52	262	—	2135	17	126	758

* Sulfamethoxypyridazine in acetate buffer; other compounds in citrate-phosphate buffer.

additional organs were weighed in most. Femoral marrows of rats were studied in undecalcified sections of the marrow pencil.[7] Bone marrows of dogs were examined in decalcified sternum sections or in sections of red femoral marrow. The Turnbull Blue reaction for iron in spleen and liver was applied in the study of anemia in dogs. Gram stain was applied to sections of some rat lesions that appeared to be caused by infection. Where sufficient data were available, statistical analyses were done using the rank method of Wilcoxon.[8, 9]

Absorption, Distribution, and Excretion in Dogs

Concentrations of SMP in blood and in cerebrospinal fluid (CSF) of dogs following single oral doses of 50, 100, and 200 mg./kg. are shown in FIGURE 2. Cumulative urinary excretion of drug following single intravenous and oral doses of SMP is shown in FIGURE 3. A urinary recovery of 60 per cent of the intravenous dose and 50 per cent of the oral doses indicates absorption of about 80 per cent for oral doses up to at least 100 mg./kg. Within experimental error, the amount of Bratton-Marshall positive material in the urine from these dogs was not increased by acid hydrolysis. As with other sulfonamides,[10] SMP was not acetylated by dogs. It would appear that a significant fraction (up to 40 per cent) of the absorbed SMP was metabolized by the dog either to compounds that did not contain the arylamine group or to compounds that gave a less intense color in the Bratton-Marshall method than an equivalent amount of the parent compound. Using the techniques of paper chromatography and countercurrent extraction on urine from dogs administered SMP, Bell *et al.*[11] found evidence that at least six different components gave a positive reaction in the Bratton-Marshall method. The major component, accounting

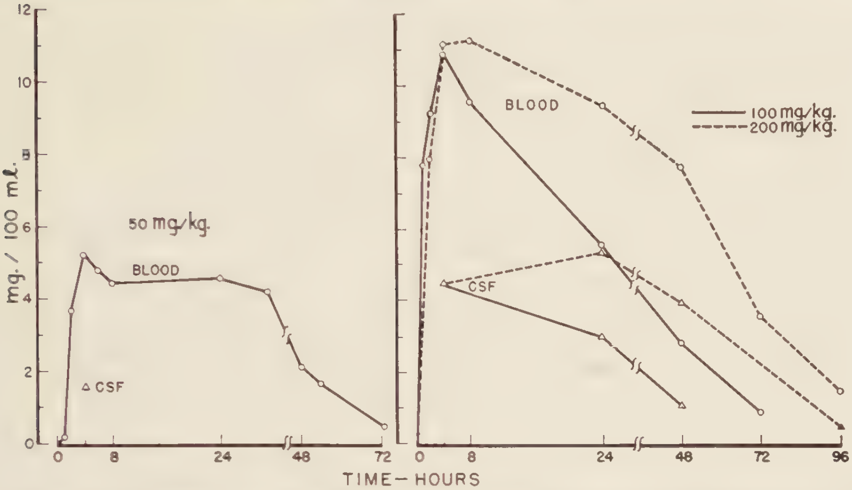

FIGURE 2. Concentrations of sulfamethoxypyridazine in blood and cerebrospinal fluid of dogs following single oral doses of 50, 100, and 200 mg./kg.

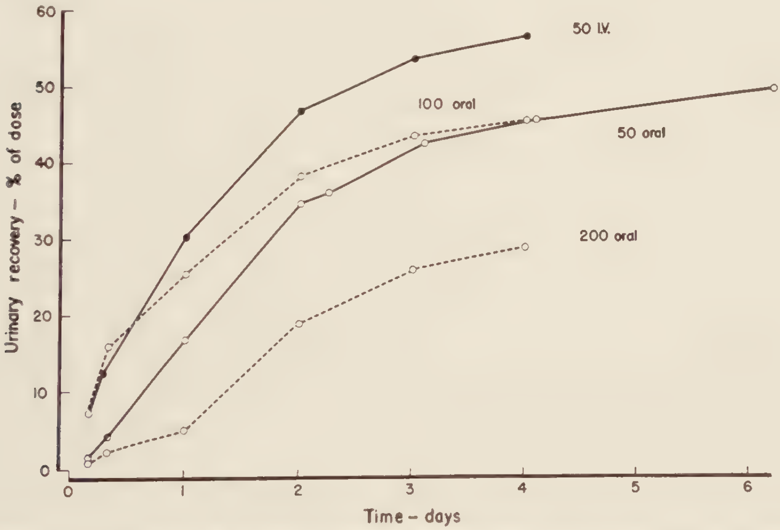

FIGURE 3. Cumulative urinary excretion of sulfamethoxypyridazine in dogs following single doses of 50 mg./kg. I. V. (K5, 12.2 kg.); 50 mg./kg. oral (K4, 15.7 kg.); 100 mg./kg. oral (K10, 11.0 kg.); and 200 mg./kg. oral (K4, 15.2 kg.).

for two thirds of the Bratton-Marshall color, appeared to be SMP and was isolated and identified as such. Another fraction appeared to be a glucuronide.

Blood concentrations of drug following an intravenous dose of SMP indicated a volume of distribution of 57 per cent and a half life in blood of 24 hr. In each of the 4 experiments with intravenous and oral doses of SMP in dogs,

TABLE 2

BINDING OF SULFAMETHOXYPYRIDAZINE BY PLASMA PROTEINS*

Concentration of drug at equilibrium—mg./100 ml.				Bound as per cent of total drug in plasma
Plasma	Dialysate	Plasma water†	Bound drug	
Plasma of dog P62				
1.91	0.57	0.54	1.37	71
20.0	9.6	9.1	10.9	54
Plasma of dog M77				
0.92	0.27	0.26	0.66	72
1.55	0.47	0.45	1.10	71
2.90	0.90	0.86	2.04	70
5.92	2.19	2.08	3.84	65
11.7	4.7	4.5	7.2	61
22.7	11.7	11.1	11.6	51

* Plasma in cellophane casing was equilibrated with buffered saline (0.15 M NaCl + 0.01 M phosphate) and SMP at pH 7.4 to 7.5 and at 25°C.
† Concentration of SMP in plasma water was calculated from concentration in dialysate on the assumption that plasma water is 95 per cent of plasma volume.

TABLE 3

DISTRIBUTION OF SULFAMETHOXYPYRIDAZINE BETWEEN PLASMA AND ERYTHROCYTES IN DOGS

Dog No. and sex	Dose* (mg./kg./day)	Hours after last dose	Hematocrit	Conc. of drug (mg./100 ml.)			Ratio of drug cells/plasma
				Whole blood	Plasma	Erythrocytes (calculated)	
K16, F	40	4	52	6.2	10.4	2.3	0.22
K22, M	40	4	50	7.4	12.1	2.7	0.22
K13, M	100	4	46	17.6	23.0	11.3	0.49
		24	44	6.1	8.6	3.0	0.35
K10, F	160	4	49	33.4	41.8	24.7	0.59
		24	46	15.0	19.7	9.5	0.48

* At the time of the test, the dogs had been on drug for periods of 8 weeks (dogs K16, K22), 22 weeks (K10), and 31 weeks (K13).

the renal clearance of the drug based on its concentration in whole blood was determined over 3 to 6 periods and averaged 2.3 ml./min. Changes in renal clearance of the drug from period to period in the same experiment, in one case as much as fourfold, appeared to be correlated to some extent with changes in pH of the urine. The degree of protein binding of SMP in dog plasma is shown in TABLE 2, and the distribution of drug between plasma and erythrocytes is shown in TABLE 3. The ratio of the concentration of SMP in erythrocytes to that in plasma of the dog was greater than that reported for man.[12] Taking into account the difference in concentration of SMP in blood and in the plasma filtrate, and assuming a glomerular filtration rate of 40 ml./min. (approximately average for our beagles), it appeared that at least 75 to 95 per cent of the drug

TABLE 4

SUMMARY OF PHARMACOLOGICAL DATA IN DOGS*

Dog No.	Compound	Renal clearance** (ml./min.)	Ratio of drug conc. (CSF/blood)	Half life in blood***		Conc. in blood after oral dose of 50 mg./kg.† (mg./100 ml.)
				Route	Hours	
K4, K5, K10	Sulfamethoxypyridazine	2.3 (0.8–4.4)	0.44 (0.32–0.54)	I. V.	24	5.3
				Oral	20 (18–22)	
P39	N⁴-Acetylsulfamethoxypyridazine	36	—	I. V.	1.5	—
K4, M79	Sulfadiazine	8 (5–10)	0.45	—	—	1.1‡
K5	Triple Sulfa§	6.4 (5–8)	0.52 (0.43–0.61)	Oral	10	4.9
M79	Sulfisoxazole	32 (29–35)	<0.1	Oral	2.5	4.8

* Values given are averages with ranges in ( ).

** Renal clearance is on a blood level basis.

*** Time for blood concentration of drug to be reduced by half. In case of oral dosage, measured from at least 20 hr. after peak blood concentration with SMP, and at least 2 hr. after peak concentration in the case of Triple Sulfa.

† Values given are maximal and refer to concentrations at 4 hr.

‡ Blood concentration of 4 to 5 mg./100 ml. at 4 hr., with oral dose of 200 mg./kg.

§ Mixture of equal parts of sulfadiazine, sulfamethazine, and sulfamerazine.

filtered at the glomeruli was reabsorbed from the renal tubules in dogs. For purposes of comparison with SMP, single experiments were carried out in dogs with oral doses of Triple Sulfa* and sulfisoxazole (50 mg./kg.) and 2 experiments with sulfadiazine (50 and 200 mg./kg.). The results are summarized in TABLE 4. The concentrations of SMP in various tissues at autopsy are given in TABLE 5. The drug does not appear to be present in any of the tissues examined at concentrations consistently higher than those in blood.

N⁴-Acetylsulfamethoxypyridazine in the Dog

N⁴-acetyl SMP was administered to one dog at doses of 100 mg./kg./day. The drug was administered orally for 7 days and intravenously (as the sodium salt) for 14 days, with an interval of 2 days between the last oral and the first intravenous dose. Oral absorption appeared to be poor or erratic. Data on blood concentrations and urinary excretion obtained during the period of intravenous administration are given in TABLE 6. Blood levels following the first intravenous dose of N⁴-acetyl SMP indicate a volume of distribution of 57 per cent and a half life in blood of 1.5 hr. The short half life is a reflection of a high renal clearance (36 ml./min.) plus a moderately rapid rate of deacetylation. From the blood concentration of free drug and its volume of distribution (57 per cent), it appears that 21 per cent of the dose was deacetylated within 3 hr. Crystals were readily seen in most of the urine samples collected during the period of intravenous administration of acetyl SMP. Analyses of the urine samples before and after filtration indicated that the

* A mixture of equal parts of sulfadiazine, sulfamerazine, and sulfamethazine.

TABLE 5

CONCENTRATIONS OF SULFAMETHOXYPYRIDAZINE IN VARIOUS TISSUES OF THE DOG FOLLOWING REPEATED DAILY ADMINISTRATION

	Dog P31, M	Dog P32, M	Dog P37, F*
Daily dose, mg./kg.	500†	250	100
Duration of treatment	2 wk.	7 wk.	27 wk.
Time between last dose and autopsy	8 days	4 days	1 day
	Conc. of drug, mg./100 gm. wet wt.‡		
Whole blood	2.4	6.0	40
Aqueous humor	1.1	—	39
Cerebrospinal fluid	1.1	—	32
Brain	1.1	2.2	22
Fat	0.6	—	8
Kidney, cortex	2.4	3.4	28
Kidney, medulla	2.5	7.4	39
Liver	4.2	5.7	30
Lung	2.3	4.1	32
Muscle	1.2	3.5	24
Spleen	—	—	26
Adrenal	—	—	28
Pancreas	—	—	27
Thyroid	—	—	32

* Dog P37 jaundiced at time of sacrifice.
† Following administration of 100 mg./kg./day for 2 weeks.
‡ Concentration in body fluids given in mg./100 ml.

TABLE 6

PHARMACOLOGY OF N[4]-ACETYLSULFAMETHOXYPYRIDAZINE IN THE DOG (100 mg./kg./day, I.V.*)

Day	Time after dose (hours)	Drug in blood (mg./100 ml.)‡			Urine + rinse**					
					Vol. (ml.)	pH (at 23°)	Drug conc. (mg./100 ml.)†			
							Unfiltered		Filtered	
		Total	Free	Acetyl			Free	Acetyl	Free	Acetyl
1	0	0	0	0						
	1	10.9	<1.5	10.9						
	2				55	6.5	18	279	18	96
	3	7.7	3.7	4.0						
	4				41	6.3	47	429	34	49
	24	1.4	1.6	0	290	—	37	60	37	33
5	4	11.2	6.4	4.8	69§	6.2	39	240	29	61
	24	2.7	3.2	0	120	6.2	115	198	110	56
8	4	9.6	6.5	3.1	11§	7.7	87	583	85	515
	24	2.7	2.9	0						
12	4	12.0	7.1	4.9	51§	7.4	61	897	58	223
	24	3.7	3.7	0	115	6.3	117	208	116	62

* Dog P39, M, wt. = 12.3 kg. Dosed days, 1 to 14.
** Volume of rinse water = 0 to 23 ml.
† Filtration at/or near room temperature (23° C.).
‡ Concentrations of free and total drug given as acetyl SMP equivalent.
§ Bladder not drained at 0 hour.

crystals consisted largely, if not entirely, of the acetyl derivative (TABLE 6). Concentrations of the acetyl drug in the urine filtered at room temperature tended to be slightly higher than the solubility of N^4-acetyl SMP at 37° C. in buffer at the corresponding *p*H (TABLE 1). This would indicate either a somewhat higher solubility of N^4-acetyl SMP in urine or the presence of metabolites other than the deacetylated drug. In addition to pronounced crystalluria, administration of acetyl SMP appeared to cause some reduction in hemoglobin (Hb) and red blood cells (RBC), 20 and 30 per cent, respectively; increases in platelets and in the sedimentation rate of blood; and an increase in reducing substances in the urine. All values returned to pre-drug levels within 3 weeks after cessation of drug administration. Other observations indicated no change in white blood cells (WBC) and differential count, urine albumin and specific gravity, blood urea nitrogen and nonprotein nitrogen (NPN), or in appetite and general behavior. There was no indication of renal damage, and the dog appeared healthy 2 years later.

Acetylation in the Rat

Concentrations of free and conjugated drug were determined in the blood and urine of rats after administration of SMP in the diet at doses of 160 mg./kg. body weight/day. The results are shown in TABLE 7. Approximately half (average = 55 per cent) of the total drug excreted in the urine is in the conjugated form (presumably N^4-acetyl SMP). This is similar to the results obtained in human subjects.[12, 13] It should be noted that under conditions approaching a steady state, that is, with constant concentrations in the blood, the ratio of conjugated to free drug in the urine is a measure of the ratio of the rate of conjugation to the rate of renal excretion of the free drug. In other words, the ratio of conjugated to free drug in the urine varies directly with the rate of conjugation and inversely with the rate of renal excretion

TABLE 7

CONJUGATION OF SULFAMETHOXYPYRIDAZINE IN THE RAT*

Rat No. & sex	Time off drug diet (hr.)	Blood: Drug conc. (mg./100 ml.)			Urine†: Collection period (hr.)	Vol. (ml.)	Drug conc. (mg./100 ml.)		Per cent conj.
		Total	Free	Conj.			Free	Conj.	
36, M	1	12.8	12.2	0.6					
59, F	1	12.2	11.7	0.5					
35, M	4		12.0		3	4	90	56	38
75, F	4		12.0		4	2	30	20	40
16, M	6		11.5		6	5	51	71	58
73, F					6	2	53	92	63
51, F	27	4.4	4.3	0.1	24	19	16	26	62
29, M	27	3.5	3.7	0	24	24	21	37	64
4, M	27	2.9	3.0	0	24	14	36	47	57
Average									55

* Rats had received SMP, 160 mg./kg. body wt./day, in diet for 2 to 3 months at the time of the tests.
† Rats taken off drug diet and put in metabolism cages at 9 A.M., with water but no food.

TABLE 8

ACUTE INTRAVENOUS TOXICITY IN MICE*

Compound	Dead/total at dose of:		
	500 mg./kg.	1000 mg./kg.	2000 mg./kg.
Sulfamethoxypyridazine	0/20	5/20	20/20
N^4-Acetylsulfamethoxypyridazine	0/10	0/10	10/10
Triple Sulfa	0/10	0/10	10/10
Sulfisoxazole	0/10	0/10	0/10

* Compounds injected in solutions of *p*H 7.5 to 11, volume = 0.2 ml./mouse; injection time = 5 sec.; observation time = 7 days. All deaths occurred within 48 hr.

of the free drug. In the case of SMP, in view of the low rate of excretion of the free drug, it was not surprising that a relatively high percentage of the drug was excreted in the conjugated form. The marked difference in blood concentrations of free and conjugated drug, with approximately equal concentrations of each in urine, showed that in the rat, as in the dog, the conjugated drug had a much higher renal clearance than the free drug. Although crystals were present in the urine collected from one of the rats (No. 35, TABLE 7), they did not appear to be composed of either the free or conjugated drugs. They were present in an alkaline urine (*p*H 7.8) and were dissolved by the addition of acid.

Acute Intravenous Toxicity in Mice and Dogs

As shown by the data given in TABLE 8,* the intravenous LD_{50} of SMP was between 1000 and 2000 mg./kg., with that for N^4-acetyl SMP and Triple Sulfa falling in the same range. Sulfisoxazole failed to kill any of 10 mice at 2000 mg./kg.

Two dogs received SMP at a relatively constant rate by intravenous infusion. In one dog (M80, M) the drug was administered at an average rate of 15 mg./kg./min. (1.1 ml./min. of a 19 per cent solution). After 65 min. of infusion with a dose of 1000 mg./kg., there were violent muscle spasms and loss of pupillary reflexes. At 90 min., with a total dose of 1350 mg./kg., the drug concentration was 159 mg./100 ml. in blood and 410 mg./100 ml. in a sample of urine (no crystals were present in the urine). The dog died after a 120-min. infusion of 1800 mg./kg. with a drug concentration of 254 mg./100 ml. in blood. No drug crystals were found in the kidney or bladder at autopsy. The second dog (P30, M) was infused at a rate of 11 mg./kg./min. The infusion was stopped at 75 min. when, with a total dose of 800 mg./kg., the dog became unconscious and showed muscle spasms. The drug concentration in blood was 105 mg./100 ml. at this time and 45 mg./100 ml. 22 hr. later. The dog appeared fully recovered by 48 hr. and appeared to be in good health 1 month later when it was sacrificed in connection with an unrelated experiment.

* We are indebted to G. S. Redin for determinations of intravenous toxicities in mice.

Chronic Toxicity in Dogs

In studies on the chronic toxicity of SMP in dogs, oral doses of 40 to 500 mg./kg./day were administered for from 2 to 33 weeks. For purposes of comparison, Triple Sulfa was administered to two dogs, one receiving up to 500 and the other 900 mg./kg./day. In the latter case the daily dose was divided, with one-third administered at 9:00 A.M., and the remainder at 4:30 P. M. each day. Observations during life covered: daily appetite, food intake, general appearance, and behavior; weekly—body weight, 4- and 24-hr. blood levels of drug, urine analyses (specific gravity, reducing substances, albumin, drug crystals, and microscopic examination of sediment), and hematology (including differential count and sedimentation rate). Blood NPN, urea nitrogen, and icteric index were determined at irregular intervals. The scope of the post-mortem studies is shown in TABLE 9, which lists the tissues selected for macroscopic and microscopic examination.

The designations, pathological and nonpathological variation, may need explanation. A pathological condition means an excessive variation from the statistical norm and/or a condition that is presumably harmful to the function of the organ. A nonpathological variation means a deviation from the norm that presumably does not interfere seriously with the function of the organ. Morphologic activation of the thyroid, an expression of functional inactivation by the drug (antithyroid effect), was classified as a nonpathological variation. This condition is reversible and can be counteracted by administration of thyroid powder. All of the sulfonamides tested for their effect on the thyroid were reported to have antithyroid action.[14]

Dogs can tolerate SMP at mean blood levels of 10 to 20 mg./100 ml. for periods of 5 months or longer with no indication of toxicity other than a possible borderline anemia. Loss of appetite and decrease in body weight occurred when around-the-clock blood levels were maintained above 20 to 25 mg./100 ml. The effect of 8 weeks' administration of SMP at 250 mg./kg./day on body weight, drug concentrations, and hemoglobin of a dog is shown in FIGURE 4. A reduction in food intake accompanied the rise in the 24-hr. drug level that occurred during the fifth week of drug administration. The dog refused all food during the last 2 weeks of drug administration. After forced feedings of meat broth for 5 days, the dog showed good recovery and appeared to be in good health 22 months after drug administration.

The results of studies on the toxicity of SMP in dogs are summarized in TABLE 10, along with the results obtained with high doses of Triple Sulfa. In no case was there any indication of drug crystals in urine samples, nor were concretions found in any part of the urinary system at autopsy. An increase in the reducing substances in the urine was observed in most cases and was assumed to reflect an increased excretion of glucuronides.[15] Toxicity associated with the higher doses of SMP (250 to 500 mg./kg./day) consisted of reduction in food intake, loss in body weight, reduction in hemoglobin and white cells, and also in liver injury. Similar changes were observed with similarly high blood levels of Triple Sulfa. Liver injury was indicated by capillary bile plugs and by a marked increase in liver weight. Two of the 3

TABLE 9

SUMMARY OF AUTOPSIES ON DOGS

A Study of Multiple Doses of Sulfamethoxypyridazine (40 to 500 mg./kg./day) and a Comparison with Triple Sulfa (250 to 500 mg./kg./day)

No., sex	Age at death years	Compound, dose, and periods of administration	Morph. proced.	Circulat.	Respirat.	Tonsils	Stomach	Jejun.-il.	Colon-coec.	Liver	Pancreas	Kidney	Ureter-bladder	Bone marrow	Spleen	Thyroid	Hypophysis	Adrenal	Reproduct.	Centr. nerv.	Nutritional state
K 21,	2	—	Ma	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	p	v	Medium
M			Mi	+	+	+				+	+	+		+	+	+	+	+	p	+	
K 14,	2½	—	Ma	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	v	+	Medium
F			Mi	+	+	+				+	+	+		+	+	+	+	+	v	+	
K 24,	2	—	Ma	+	v	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Medium
F			Mi	+	+					+	+	+		+	+	+	+	+	+	+	
K 22,	2	SMP 40 mg. for 10 wk.	Ma	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	Medium
M			Mi	+	+					+	+	+		+	+	v	+	+	+	+	
K 16,	2½	SMP 40 mg. for 10 wk.	Ma	+	+	+	+	+	+	+	+	+	+	+	+	v	+	+	+	+	Obese
F			Mi	+	+	+				+	+	v		+	+	v		+	+	+	
P 37,	4	SMP 100 mg. for 27 wk.	Ma	+	+		+	+	+	+	+	+	+	+	+	v	+	+	+	p	Medium
F			Mi	+	p			+		v	+	+		v	p	v		+		p	
P 32,	4½	SMP 250 mg. for 7 wk.	Ma	+	+		+	+	+	p	+	+	+	+	+	+	+	+	+	v	Lean
M			Mi	+	+					p		p			p	v	+	v	p	+	
P 31,	4½	SMP 100 mg. for 2 wk.	Ma	+	+		+	+	+	p		+	+	+	+	v		+	+	+	Lean
M		500 mg. for 2 wk.	Mi	+	p					p		v			p	v		v	+	+	
K 2,	3	Triple Sulfa 250 mg. for 7 wk.	Ma	+	+		+	+	+	p	+	p	+	v	+			+	p	+	Very lean
M		500 mg. for 5 wk.	Mi	+	p					p	+	p		v	p			+	p	+	
K 13,	2½	SMP 100 mg. for 33 wk.	Ma	+	+		+	+	+	+	+	+	+	+	+	v	+	+	p	+	Medium
M		no drug for 21 wk.*	Mi		+					+		+		+	+	v			p		
K 10,	3	SMP 160 mg. for 22 wk.	Ma	+	+		+	+	+	+	+	+	+	+	+	v	+	+	+	+	Obese
F		no drug for 21 wk.*	Mi		+					+		+		+	+	v					

Symbols: Ma = macroscopic; Mi = microscopic; + = normal; v = nonpathological variation; p = pathological; blank = not examined.

* In other words, there was an interval of 21 weeks between the last dose and sacrifice.

TABLE 9 SUPPLEMENT

Dog K 21, M
Reproduct.: prostate; suppurative inflammation.
Centr. nerv.: slight internal hydrocephalus.

Dog K 14, F
Reproduct.: ovary; cystic corpora lutea.

Dog K 24, F
Respirat.: small subpleural nodules, possible parasitic (not found in the microscopic sections).

Dog K 22, M
Thyroid: absolute weight 2.0 gm., indications of slight morphologic activation.

Dog K 16, F
Kidney: lymphocytic infiltrations in wall of pelves and in the adventitia of some blood vessels.
Thyroid: absolute weight 2.4 gm.; morphologic activation of highest degree.

Dog P 37, F
Respirat.: congestion and edema.
Liver: weight normal; granular bile pigment in liver cord cells; iron reaction ++ in Kupffer cells.
Spleen: iron reaction +++; atrophy of lymph nodules.
Thyroid: morphologic activation +++.
Centr. nerv.: moderate hydrocephalus; possibly increased subependymal cellularity.
Bone marrow: sternum—hematopoietic tissue normal in composition but small in amount; femur—fat tissue.

Dog P 32, M
Liver: weight greatly increased; capillary bile plugs more numerous than in Dog P 31; iron reaction +++ in Kupffer cells; general jaundice of the dog.
Kidney: small amount of bile pigment.
Spleen: iron reaction ++++; atrophy of lymph nodules.
Thyroid: morphologic activation +++; no increase in volume or relative weight.
Adrenal: visible cortical lipids decreased?
Reproduct.: in some testicular tubules normal spermatogenesis; abnormal sloughing off of spermatogenic cells in others.
Centr. nerv.: slight ventricular dilatation.

Dog P 31, M
Respirat.: small foci of granulation tissue.
Liver: weight greatly increased; capillary bile plugs; iron reaction + in Kupffer cells.
Kidney: protrusion of tubular epithelium into space of Bowman's capsule (innocuous variation in dogs).
Spleen: iron reaction ++.
Thyroid: morphologic activation ++; with hyperemia, but without increase in volume or relative weight.
Adrenal: visible cortical lipids decreased?

Dog K 2, M—slight post-mortem autolysis.
Conjunctival and costal cartilage: jaundiced.
Respirat.: congestion and edema.
Liver: weight greatly increased; capillary bile plugs; possibly small necrotic foci and granulomas; iron reaction + in Kupffer cells.
Kidney: enlargement of cortex; lack of tubular fat in transitional zone, abnormal presence in cortex and medulla; protrusion of tubular epithelium into space of Bowman's capsule (innocuous variation in dogs).
Spleen: iron reaction +++; atrophy of lymph nodules.
Reproduct.: hypoplasia or atrophy of right testis; normal spermatogenesis in left testis.
Bone marrow: femur—subcortical layer of hematopoietic tissue.

Dog K 13, M
Thyroid: absolute weight 11.3 gm.;* colloid goiter of moderate size; accessory thyroid nodules in storage phase.
Reproduct.: left testis ectopic in left groin, no spermatogenesis; right testis in scrotum, normal spermatogenesis.

Dog K 10, F
Thyroid: absolute weight 5.9 gm.;* colloid goiter of moderate size.

* Absolute thyroid weights of control dogs = 1.1 to 1.4 gm.

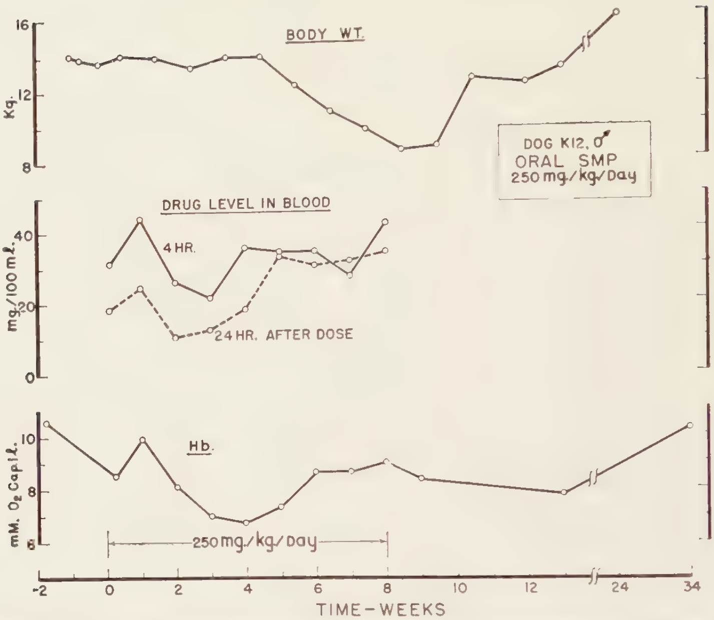

FIGURE 4. Study of 8-week administration of sulfamethoxypyridazine at 250 mg./kg./day in dog K12: body weight, drug levels, and hemoglobin (normal hemoglobin values for beagles range from 8 to 10.5 mM O_2 cap./l. equivalent to 14 to 18 gm. Hb/100 ml.).

dogs that showed liver injury (P32 and K2) were jaundiced at death. Evidence of an antithyroid action of SMP was seen at all dosage levels. In dogs K13 and K10, taken off drug for 21 weeks before autopsy, the thyroids showed a storage phase. This may be expected to occur subsequent to a period of morphologic activation.[16]

Chronic Toxicity in Rats

SMP was administered in the diet to growing rats at doses of 80, 160, and 320 mg./kg. body weight/day for a period of 7 months. The rats were 5 to 6 weeks of age (average weight, 88 gm.) at the start of drug administration. They were housed in individual cages. Individual food and drug intakes and body weights were determined weekly. Drug concentrations in the diet were adjusted biweekly to maintain the scheduled average drug intake for each sex group. Blood samples were taken from one male and one female of each group once each month for hematology and for determination of free drug. Skeletal lengths, from the tip of the nose to the base of the tail, were measured after 3 weeks on drug and again at 7 months. The scope of the post-mortem studies was similar to that of those done on dogs (TABLE 9). With a majority of the rats, however, the autopsies were abbreviated, with fewer organs weighed and examined microscopically.*

The results of the study in rats are summarized in TABLE 11. Urinary

* Detailed protocols of the studies in both rats and dogs will be supplied on request.

Table 10

Evaluation of Results of Studies of Sulfamethoxypyridazine in Dogs

	Controls (no drug) K 21, M K 14, F K 24, F	Sulfamethoxypyridazine (mg./kg./day) 40 mg. 10 wk. K 16, F K 22, M	100 mg. 27 wk. P 37, F	100 mg. 33 wk. (followed by 21 wk. without drug) K 13, M	160 mg. 22 wk. (followed by 21 wk. without drug) K 10, F	250 mg. 7–8 wk. P 32, M K 12, M	500 mg. 2 wk. (after 100 mg. for 2 wk.) P 31, M	Triple Sulfa (mg./kg./day) 500 mg. 5 wk. (after 250 mg. for 7 wk.) K 2, M	900 mg. 4 wk. K 3, M
Drug conc. in blood*									
At 4 hr.	–	6.2	36**	13	21	33–40***	50†	45††	72
At 24 hr.	—	2.3	26**	6	10	28–33***	46†	36††	65
Deaths/total	0/3	0/2	0/1	0/1	0/1	1/2†††	0/1	1/1	1/1
Crystalluria	0	0	0	0	0	0	0	0	0
Concretions in urinary system	0	0	0	0	0	0	0	0	0
Food intake	Norm.	Norm.	Decr.	Norm.	Norm.	Decr.	Decr.	Decr.	Decr.
Body weight	Norm.	Norm.	Decr.	Norm.	Norm.	Decr.	Decr.	Decr.	Decr.
Hemoglobin	Norm.	Norm.	Slightly decr.	Slightly decr. ?	Slightly decr. ?	Decr. (P 32, M) Slightly decr. (K 12, M)	Norm.	Decr.	Norm.‡
White blood cells	Norm.	Norm.	Slightly decr.	Norm.	Norm.	Decr.	Decr.	Decr.	Incr.‡
Liver injury	0	0	0	0	0	Present (P 32, M)†††	Present	Present	Not determined
Morphol. activation of thyroid, including sequelae	0	Marked (K 16, F); Slight (K 22, M)	Marked	§	§	Marked (P 32, M)	Moderate	Not determined	Not determined

* Mg. drug/100 ml. blood; averages of weekly determinations.
** Values entered are of last 6 wk.
*** Values entered are of last 4 wk.
† Values entered are of last 2 wk.
†† Values entered are of last 3 wk.
††† One sacrificed moribund (P 32, M), listed as death. K 12, M still alive 20 mo. after drug treatment.
‡ Dog dehydrated.
§ Extreme storage phase after a drugless period of 21 wk.

Table 11

Evaluation of Results of 30 Weeks' Administration of Sulfamethoxypyridazine in Young Rats

Six Males and 6 Females in Each Group; Doses mg./kg./day

	I Controls (no drug)	II 80 mg.	III 160 mg.	IV 320 mg.
Drug concentration in blood*	—	9.5	14	18
Concretions in urinary system	0	7/12**	5/12**	2/9**
Deaths/total	0/12	0/12	0/12	9/12†
Food intake	Satisfactory	As controls	Males decreased, females as controls	Decreased
Body weight gain	Satisfactory	As controls	Males decreased, females as controls	Decreased
Skeletal and femoral length	Satisfactory	As controls	Shorter than controls	Not determined
Hemoglobin	Satisfactory	Males as controls, females sl. decr.	Sl. decreased	Decreased
White blood cells	Satisfactory	Males decreased, females as controls	Decreased	Decreased
Lesions caused by infections	1	1	0	7‡
Tumors	0	0	0	1(?)‡
Morphol. activation of thyroid, including sequelae	0§	9	11	5‡

*Mg. drug/100 ml. blood; averages of whole period of drug administration.
**Concretions from one rat of each group were examined crystallographically; no evidence of sulfamethoxypyridazine or its N^4-acetylated derivative was found.
† One sacrificed moribund, listed as death.
‡ Three rats of Group IV found dead were not autopsied.
§ Slight activation as usual at 74° F. environmental temperature.

concretions were found in rats of each of the 3 drug-treated groups, but not in the control group. The incidence of concretions appeared to vary in an inverse manner with the dose of drug. In all cases the concretions were in the renal pelves, and in 3 cases were also in the bladder. Crystallographic examinations of samples of concretions from one rat of each drug-treated group gave no indication that these were composed mainly of SMP or N^4-acetyl SMP. The concretions were not appreciably soluble in dilute ammonia, and a high ash content indicated that they were essentially inorganic in nature.* The blood concentrations of drug associated with a reduction in food intake and body weight, 14 to 18 mg. 100 ml., are similar to those reported by Schmidt *et al.*[17] for sulfadiazine, sulfamerazine, and sulfamethazine, and by Randall *et al.*[18] for sulfisoxazole. The incidence of lesions caused by infection

* We are indebted to A. F. Kirkpatrick for the crystallographic and microchemical tests on the urinary concretions.

was so high in the group of rats on the high dose of SMP that a lowering of resistance, possibly secondary to the reduced food intake, must be assumed. A subcutaneous node occurred in one rat of the highest dose group. The node was histologically on the border line of granuloma and sarcoma. This was the only possible tumor in the 33 drug-treated rats autopsied. As was the case in dogs, morphologic activation of the thyroid was observed in some rats at all dosage levels of SMP.

Summary

In the dog, sulfamethoxypyridazine was well absorbed orally and gave prolonged blood levels as a result of slow renal excretion. The ratio of its concentrations in cerebrospinal fluid and blood was similar to that obtained for sulfadiazine and Triple Sulfa. Protein binding of sulfamethoxypyridazine in dog plasma ranged from 71 per cent at a drug concentration of 1 mg./100 ml. plasma to 51 per cent at 23 mg./100 ml.

The renal clearance of N^4-acetylsulfamethoxypyridazine in the dog was appreciably higher than that of the free drug; a similar relationship between the free and conjugated drug existed in the rat. Sulfamethoxypyridazine was excreted slowly also in the rat, which conjugated (presumably acetylated) and excreted the free drug at approximately equal rates.

Chronic toxicity studies in dogs and rats indicate that, on a blood-level basis, the toxicity of sulfamethoxypyridazine is similar to that of other antibacterial sulfonamides in current use.

Acknowledgment

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SULFAMETHOXYPYRIDAZINE AND SULFACHLOROPYRIDAZINE*

By Wilfred F. Jones and Maxwell Finland

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The potential advantages of long-acting antimicrobial agents are self-evident, particularly if they are effective in the prophylaxis or in the prolonged treatment of subacute and chronic infections or in the suppression of chronic infections that are difficult to eradicate. The new antibacterial sulfonamide, sulfamethoxypyridazine (3-sulfanilamido-6-methoxypyridazine),† as noted elsewhere in these pages by Roepke and his co-workers, was found to have, as judged from experiments in animals, the following desirable properties: high solubility in urine, good absorption from the gastrointestinal tract, very slow urinary excretion, poor acetylation in the blood, good penetration into the brain and cerebrospinal fluid, and high antibacterial action about equal to that of sulfadiazine and, according to data presented by Litchfield,[1] superior to that of other popular sulfonamide drugs. These properties were of sufficient interest to warrant exploration of the action of this agent in humans.

Preliminary observations on absorption and excretion of sulfamethoxypyridazine in man were carried out by Nichols *et al.*[2] These investigators gave a single oral dose of 4 gm. to each of 6 normal adult males and determined concentrations in the blood and urine. The dose was well absorbed, yielding in every subject peak levels of over 20 mg. of the free drug per 100 ml. of plasma between 5 and 12 hr. after the dose, with only small amounts in the conjugated form. Little, if any, of the drug diffused into the blood cells. The free drug was cleared slowly from the plasma, the conjugated form being cleared, on the average, about 11 times as fast as the free form.

Concentrations of the drug in the urine after this dose, with uncontrolled fluid intake, ranged up to 200 mg. per 100 ml.; between 35 and 60 per cent of this amount was shown to be in the conjugated form. Significant levels were still present in the blood and urine 105 hr. after the dose. Less than one half of the administered dose was recovered in the urine within 48 hr. after it had been ingested. The only untoward effect noted was lassitude and frontal headache in 3 of the subjects during the period of the peak concentrations of the drug in the blood, namely, from 4 to 8 hr. after the dose was taken.

Additional and more complete studies on the absorption and excretion of sulfamethoxypyridazine were reported by Nichols and Finland.[3] After a single oral dose of 3 gm., maximum levels of nearly 26 mg. per 100 ml. were demonstrated in the plasma within about 7 hr.; these levels fell gradually to insignificant concentrations over a period of 8 days or longer. Nearly all of the administered drug was recovered in the urine, where detectable amounts

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† Supplied by Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y., under the registered name Kynex.

were still demonstrable 10 to 14 days after the dose. Between 10 and 15 per cent of the drug in the plasma and more than one half of that in the urine were found in the conjugated form.

Some attempts were made to influence the rate of excretion of the drug by various means. Diuresis resulting from increased intake of fluid had no appreciable effect. The oral administration of alkalis for brief periods had only a slight effect in increasing the rate of excretion. A definite, although slight and brief, increase in its clearance from the plasma accompanied the diuresis and alkalinization of the urine, which followed the administration of 0.5 gm. of acetazolamide (Diamox).

Preliminary observations were made in 7 patients with urinary tract infections who had been given 1 gm. orally every 48 hr. This dosage produced average plasma levels ranging between 5 and 12.5 mg. of the total sulfamethoxypyridazine per 100 ml., and also produced significant concentrations in the urine in the intervals between doses. The mean peak values in the plasma following the first few doses increased gradually until 6 doses had been given, but there were no further increases in the peak levels thereafter. There was a greater cumulative effect in one patient who suffered from impaired renal function, and the therapy was discontinued after 5 doses, although the nonprotein nitrogen in the blood of this patient declined progressively and the patient improved during this time.

The only untoward effects noted by Nichols and Finland were some lassitude and headache from 4 to 8 hr. after the dose in one half the subjects who received the single 3-gm. dose. No untoward effects were experienced in the patients to whom repeated doses of 1 gm. were given every 48 hr.

Frisk and Wassen[4] reported a clinical evaluation of sulfamethoxypyridazine at the Fourth Annual Symposium on Antibiotics. After a single oral dose of 4 gm. in 6 patients, they obtained mean peak concentrations of about 19 mg. per 100 ml. of whole blood at 4 and 8 hr.; of this amount only about 5 per cent was conjugated. Ninety-six hr. after the dose, the level in the whole blood was 3.3 mg. per 100 ml., of which 2.9 mg. was in the free form. The urine of these patients contained up to 140 mg. per 100 ml. of the drug and only rarely exceeded 200 mg. per 100 ml. Between 20 and 35 per cent of these amounts were in the conjugated form. Only about two thirds of the administered dose was excreted during the first 96 hr.

These authors also presented data on concentrations of sulfamethoxypyridazine in the blood during continuous administration of 1 gm. every 12 hr. During the first 5 days they observed a tendency for the concentrations of the drug to cumulate, but thereafter the concentration was maintained at a fixed level of about 17 mg. per 100 ml. of whole blood; about 1.5 to 2 mg. of this amount was in the conjugated form. No crystals were detected in the urine and no disturbance of renal function was elicited, but it was felt that these doses were excessive and that 1 gm. daily was probably optimal.

In the same paper, Frisk and Wassen also reported the results of treatment of 22 patients with pyelonephritis with sulfamethoxypyridazine; 4 patients in whom renal function had been appreciably reduced were included in their study. They gave a dose of 1 gm. every 12 hr., usually for 5 to 6 days; the

largest total dose was 24 gm. Clinical improvement and sterile urine were achieved in 10 patients; some improvement in the clinical symptoms and in the urinary findings was noted in 7 others. In 3 of 6 patients with infections due to *Escherichia coli* in whom some improvement was noted, the *E. coli* disappeared from the urine, and sulfonamide-resistant strains of *Streptococcus fecalis* were subsequently cultivated from the urine. The drug was well tolerated by these patients. Only one patient experienced slight nausea; another developed fever and a rash. No disturbances of the blood or of renal function were encountered.

Boger *et al.*[5] studied the absorption and excretion of sulfamethoxypyridazine in 67 patients and administered it to 35 additional patients in doses ranging from 1 to 4 gm. They noted that the drug was absorbed rapidly from the gastrointestinal tract, and that therapeutically significant plasma concentrations were promptly achieved and then maintained for many hours. They could measure the drug in the plasma for as long as 168 hr. after a single 2-gm. dose. They accounted for 20 to 30 per cent of the administered drug in the urine within 24 hr., and from 40 to 50 per cent within 48 hr., the acetylated compound constituting almost one half of the drug excreted in the urine in nearly every case. They therefore considered the solubility of the acetylated compound in the urine to be critical so far as potential crystalluria in connection with this drug was concerned. From this point of view the good solubility in water of the N^1 acetylated form, which accounts for most of the conjugated drug found in the urine, is important.[6] Boger *et al.*[5] also studied the diffusion of sulfamethoxypyridazine into the cerebrospinal fluid of patients with normal meninges and found this to occur to a greater extent than with other commonly employed sulfonamides.

Foerster *et al.*[7] compared the blood concentrations of sulfamethoxypyridazine with those of sulfaethylthiadiazole and sulfadiazine after oral administration of single doses of 2 gm. of these respective drugs. Of the three compounds, sulfamethoxypyridazine yielded the highest mean concentrations in the blood at each interval studied. The concentration of sulfamethoxypyridazine at 48 hr. approximated the 12-hr. concentration of sulfaethylthiadiazole and the 6-hr. concentration of sulfadiazine.

In the present paper we wish to present further observations on sulfamethoxypyridazine on different dosage regimens. In addition, we shall present some preliminary observations on another related compound, sulfachloropyridazine (Ba-10370),* the latter being identical in structure, except that the OCH_3 on the pyridazine is replaced by Cl. The two compounds will be compared with respect to blood levels and urinary excretion. Some comparisons of the properties of these two compounds and of their effects *in vitro* and in animals are presented by Neipp and Mayer elsewhere in this monograph.

Results

Blood levels after a single oral dose. A comparison of plasma levels of sulfamethoxypyridazine and sulfachloropyridazine is seen best in the upper panels

* Furnished by CIBA Pharmaceutical Products Inc., Summit, N. J.

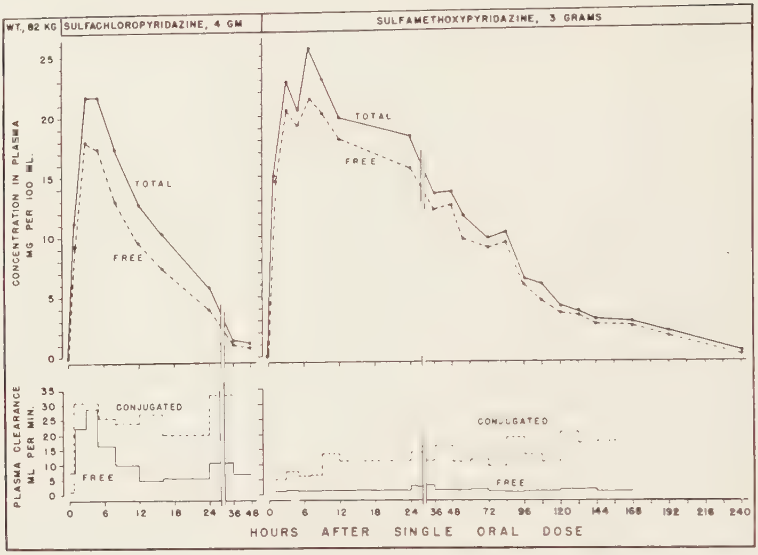

FIGURE 1. Concentrations in the plasma and plasma clearances of two sulfapyridazines in the same normal subject.

of FIGURE 1, which depict the values obtained after a single dose of 4 gm. of the former and 3 gm. of the latter given orally to the same normal adult male, who weighed 82 kg. The peak level of sulfachloropyridazine after the 4-gm. oral dose (left panel) was about 21.7 mg. per 100 ml., of which 18.0 mg. was in the free form; this was achieved in 3 hr. The corresponding concentrations in the whole blood (not shown in the figure) at this time were 12.1 and 10.7 mg. per 100 ml., respectively. Thereafter the levels declined quite rapidly to 5.8 mg. total and 4.0 mg. free per 100 ml. of plasma at 24 hr., and to insignificant levels at 48 hr. The peak level after 3 gm. of sulfamethoxypyridazine (right panel) was 25.6 mg. per 100 ml. of plasma, of which 21.4 mg. was in the free form; this peak was achieved 7 hr. after the dose, and the concentrations declined very slowly so that the level in the plasma at 24 hr. was 18.2 mg. per 100 ml., of which 15.6 mg. was free drug. At 48 hr. the corresponding levels were 13.6 mg. and 12.5 mg., respectively; at 144 hr. after ingestion of the dose they were 2.9 mg. and 2.5 mg., respectively.

Clearances. The plasma clearances, estimated without correction for the protein binding, are shown in the lower panels. As could be expected from the plasma levels, the sulfachloropyridazine was cleared from the plasma much more rapidly than was sulfamethoxypyridazine. The difference was most striking for the free drug during the period when the plasma levels were highest.

Urinary excretion of single doses: concentrations in urine. Even more striking were the differences in the urinary excretion of the two compounds (FIGURE 2). The upper panels of this figure show the concentrations of free and conjugated drug found in the urine collected at various intervals after a single dose of 4 gm. of sulfachloropyridazine (on the left) and 3 gm. of sulfamethoxypyrida-

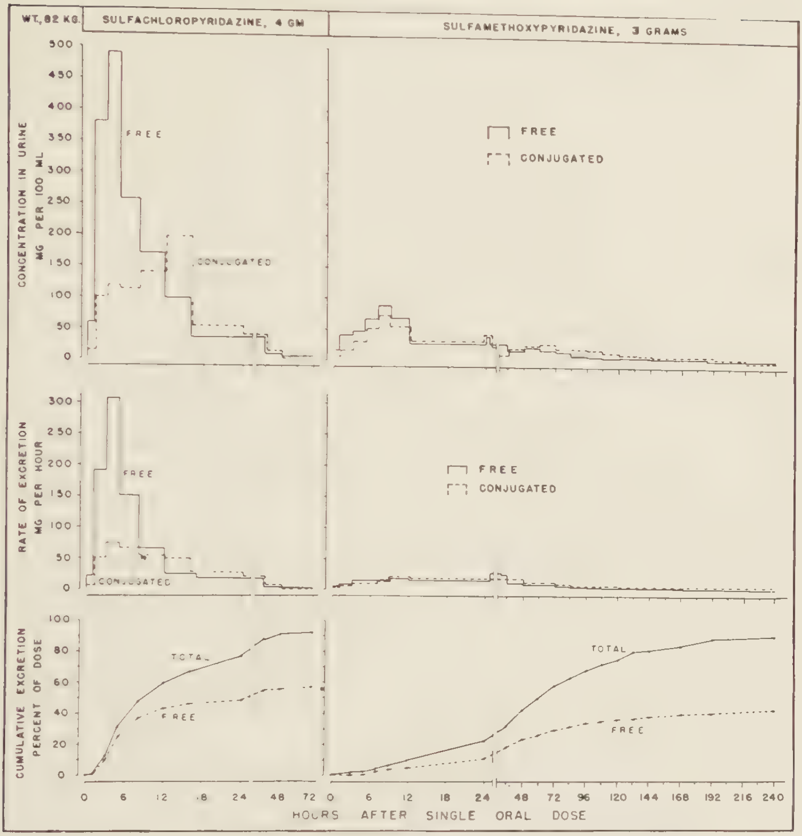

FIGURE 2. Urinary excretion of two sulfapyridazines in the same normal subject.

zine (on the right). The urine voided by this subject in the interval between 1 and 3 hr. after the 4-gm. dose of sulfachloropyridazine contained 480 mg. per 100 ml., of which 380 mg. was in the free form. The concentrations in the urine voided during the following 2 hr. in this subject was 608 mg. per 100 ml., of which 490 mg. was in the free form. In a second subject who received a similar dose and was putting out a slightly smaller volume of urine (not shown in this figure), the concentration in the urine excreted between 1 and 3 hr. was 616 mg. per 100 ml., of which 463 mg. was in the free form; the corresponding concentrations in the urine excreted between 3 and 5 hr. were 667 and 502 mg. per 100 ml., respectively. These values are in contrast to those depicted after the 3-gm. dose of sulfamethoxypyridazine in FIGURE 2. The highest concentration in the urine after this dose was achieved between 7 and 9 hr. and was 164 mg. per 100 ml., of which 88 mg. was in the free form. In another study the same subject was given 4 gm. of sulfamethoxypyridazine, and the highest concentration of drug demonstrated in the urine was between 8 and 12 hr. after this dose and was 105 mg. per 100 ml., of which 64 mg. was in the free form.

Rate of excretion. The values just cited were, of course, dependent on the volume of urine excreted. The rate of excretion of the drug is depicted in the middle panels of FIGURE 2. Between the third and the fifth hour after ingestion of 4 gm. of sulfachloropyridazine, when excretion was maximum, the drug was excreted at a rate of 380 mg. per hour; of this amount 306 mg. was free drug and 74 mg. was in a conjugated form. This contrasts with a maximum rate of excretion of sulfamethoxypyridazine that occurred between 9 and 12 hr. and was 36 mg. per hour, of which 19 mg. was conjugated and 17 mg. was in the free form. In the earlier study in the same subject, after he received 4 gm. of sulfamethoxypyridazine, the maximum urinary excretion was observed between 12 and 16 hr. after the dose and was also at the rate of 36 mg. per hour, half of which was in the free form. In a second subject, who received 4 gm. of sulfachloropyridazine, the maximum rate of excretion, which occurred between 3 and 5 hr., was 367 mg. per hour, of which 276 mg. was free and 91 mg. was conjugated. Thus, the maximum rate of excretion of sulfachloropyridazine was about 10 times as rapid as that of sulfamethoxypyridazine.

Urinary recoveries. The lowest panels in FIGURE 2 depict the cumulative amounts of drugs recovered in the urine, shown as cumulative per cent of the administered dose excreted. Almost all of each drug was accounted for in the urine; a total of 92 per cent of the 4-gm. dose of sulfachloropyridazine was accounted for within 48 hr., and only insignificant amounts totaling 1 per cent of the dose could be demonstrated in the urine during the next 4 days. In contrast, only 89 per cent of the 3-gm. dose of sulfamethoxypyridazine had been accounted for by the end of the tenth day, and the drug was still being excreted in appreciable amounts 4 days later.

Estimation of "half life." The half life of a single oral dose of the two drugs has been estimated in various ways from the data available; the results are shown in TABLE 1. The half life in the plasma, estimated as the time required to reach one half of the peak concentration in the plasma, was about 5 times

TABLE 1

ESTIMATED "HALF LIFE" OF SULFAMETHOXYPYRIDAZINE AND SULFACHLOROPYRIDAZINE AFTER A SINGLE ORAL DOSE IN NORMAL ADULT MEN

Definition of half life	Drug*	Dose (gm.)	Half life in hours			Number of subjects
			Maximum	Minimum	Mean	
Hours required to achieve one half of maximum concentration in plasma	SMP	4	60	46	53	4
	SMP	3	66	47	58	7
	SCP	4	12½	10½	11½	2
Hours required to excrete one half of administered dose into the urine	SMP	4	72	44	60	4
	SMP	3	61	48	59	7
	SCP	4	9	8	8½	2
Hours required to excrete one half of the total amount recovered in the urine	SMP	3	58	37	50	6
	SCP	4	7	6	6¾	2

* Symbols: SMP = sulfamethoxypyridazine; SCP = sulfachloropyridazine.

as long for sulfamethoxypyridazine as for sulfachloropyridazine. In the subject whose data are depicted in FIGURE 1, the half life in the plasma was 12½ hr. after the 4-gm. oral dose of sulfachloropyridazine, about 54 hr. after 3 gm. of sulfamethoxypyridazine, and about 60 hr. after 4 gm. of the latter drug in another study.

The half life of the drugs in the body was estimated on the basis of excretion into the urine. If complete elimination of each drug in the urine was assumed, the half life in the body could be considered to be the time required to demonstrate one half of the administered dose in the urine. For the subject whose findings are shown in FIGURE 2, the half life for the 4-gm. dose of sulfachloropyridazine was about 9 hr., the half life of the 3-gm. dose of sulfamethoxypyridazine was 60 hr. and, when he was given 4 gm. of sulfamethoxypyridazine in another study, it was 72 hr. Calculated on the basis of the time required to excrete one half of the total amount actually recovered in the urine, the half life was somewhat shorter. By either criterion, the half life of sulfamethoxypyridazine in the body was about 7 or 7½ times as long as that of sulfachloropyridazine.

Concentration of sulfamethoxypyridazine and sulfachloropyridazine in blood cells. A number of specimens of blood obtained from individuals receiving a single dose of either sulfamethoxypyridazine or sulfachloropyridazine were assayed for their content of the drugs in whole (oxalated) blood and in the plasmas of the same specimens. Except in occasional specimens with high concentrations of these drugs, all of the sulfonamide in the whole blood could be accounted for in the plasma, and none could be detected in the cellular elements.

Concentrations of sulfamethoxypyridazine and sulfachloropyridazine in blood and urine during the administration of repeated oral doses. Six patients were given 0.5 gm. of sulfamethoxypyridazine every 12 hr., and blood samples were obtained before, and 5 and 12 hr. after the first, fifth, and thirteenth (or fifteenth) dose; additional blood samples were drawn 2 and 8 hr. after the first dose. The concentrations of total and free sulfamethoxypyridazine in these specimens of whole blood are summarized in TABLE 2. There was a steady increase in the concentration of drug as treatment continued, but the fluctuations in the concentrations between doses tended to level off. Also, the proportion of the total drug found in the conjugated form remained small; it averaged about 16 per cent after the first and fifth doses, and 21 per cent after the thirteenth or fifteenth dose. The levels in the plasmas of these samples were nearly twice as high as those in the whole blood.

Concentrations of sulfamethoxypyridazine in random specimens of urine obtained on different days after the onset of treatment with 0.5 gm. every 12 hr. are shown in TABLE 3. There were wide fluctuations in these concentrations in the urine, with a general upward trend as treatment continued. The proportion of drug demonstrated in the urine in the conjugated form varied widely also among the different patients, but the variations appeared to be less marked in the same patient from day to day. On the average, the conjugated form accounted for from one half to about four fifths of the total amount found in the urine.

TABLE 2

CONCENTRATIONS OF SULFAMETHOXYPYRIDAZINE IN WHOLE BLOOD DURING ORAL ADMINISTRATION OF ONE-HALF GRAM EVERY 12 HOURS

Hours after previous dose	Sulfamethoxypyridazine, mg./100 ml. (Average ± S.D.)*		
	Day 1 (dose 1)	Day 3 (dose 5)	Day 7 or 8 (dose 13 or 15)
Total			
0 (or 12)	0	4.9 ± 0.7	7.6 ± 1.3
2	1.8 ± 1.2		
5	2.5 ± 0.3	6.0 ± 1.2	7.9 ± 0.5
8	2.4 ± 0.5		
12	2.1 ± 0.4	5.5 ± 0.9	7.7 ± 0.7
Free			
0	0	3.9 ± 0.8	6.3 ± 1.4
2	1.4 ± 0.9		
5	2.1 ± 0.5	5.2 ± 1.2	6.0 ± 1.2
8	2.1 ± 0.4		
12	1.8 ± 0.4	4.6 ± 0.8	6.0 ± 1.0

* Six patients on days 1 and 3; 5 patients on day 7 or 8.

TABLE 3

CONCENTRATIONS OF SULFAMETHOXYPYRIDAZINE IN RANDOM SPECIMENS OF URINE OF PATIENTS RECEIVING ONE-HALF GRAM EVERY 12 HOURS

Day	Number of Specimens	Sulfamethoxypyridazine in urine (mg./100 ml.)					
		Total		Free		Per cent conjugated	
		High	Low	High	Low	High	Low
1	5	123	8	21	4	85	49
3	5	313	19	50	4	84	52
6	4	363	56	50	25	86	55
8	4	152	63	57	29	67	54

Five patients were given sulfachloropyridazine orally in doses of 1 gm. every 6 hr. The levels in the whole blood obtained 1, 2, and 6 hr. after the first dose on days 1, 2, 4, and 7 are shown in TABLE 4. Here there was an increase in concentrations through the fourth day, but the concentrations were lower on the seventh day. There were moderate fluctuations in the levels between doses throughout the 7 days. The proportion of the drug determined in the conjugated form tended to increase somewhat; this increase averaged 20 per cent on the first day, 26 per cent on the second and fourth days, and 32 per cent on the seventh day. The blood levels obtained after 4 gm. a day of sulfachloropyridazine were of about the same order of magnitude as those obtained with 1 gm. daily of sulfamethoxypyridazine.

Concentrations of sulfachloropyridazine in random specimens of urine in these 5 patients who were given 1 gm. every 6 hr. are summarized in TABLE 5.

TABLE 4
CONCENTRATIONS OF SULFACHLOROPYRIDAZINE IN WHOLE BLOOD OF 5 PATIENTS DURING ADMINISTRATION OF 1 GRAM ORALLY EVERY 6 HOURS

Hours after previous dose	Sulfachloropyridazine, mg./100 ml. (Average ± S.D.)			
	Day 1 (dose 1)	Day 2 (dose 5)	Day 4 (dose 13)	Day 7 (dose 25)
	Total			
1	2.7 ± 1.5	5.8 ± 0.9	7.2 ± 1.6	5.3 ± 1.5
2	4.2 ± 1.2	6.8 ± 1.0	7.7 ± 1.8	4.7 ± 1.4
6	2.8 ± 0.4	5.9 ± 1.6	5.4 ± 2.0	3.0 ± 1.5
	Free			
1	2.3 ± 1.5	4.0 ± 0.5	5.5 ± 1.8	3.7 ± 1.1
2	3.3 ± 0.7	5.3 ± 1.0	5.6 ± 1.4	3.1 ± 0.7
6	2.1 ± 0.3	4.5 ± 1.2	4.0 ± 1.3	2.0 ± 0.9

TABLE 5
CONCENTRATIONS OF SULFACHLOROPYRIDAZINE IN RANDOM SPECIMENS OF URINE OF 5 PATIENTS RECEIVING 1 GRAM ORALLY EVERY 6 HOURS

Day*	Sulfachloropyridazine in urine (mg./100 ml.)					
	Total		Free		Per cent conjugated	
	High	Low	High	Low	High	Low
1	248	26	152	14	60	36
2	360	100	188	35	65	43
4	712	106	419	42	61	39
7	738	100	481	56	73	35

* One specimen of urine was obtained from each patient on each of these days.

These concentrations were about twice as high as those obtained during the administration of 0.5 gm. of sulfamethoxypyridazine every 12 hr. The proportions of drug found in the urine in the conjugated form were less during the sulfachloropyridazine administration than when sulfamethoxypyridazine was given; as a consequence, the concentrations of free sulfachloropyridazine in the urine were several times as high as those with sulfamethoxypyridazine.

Preliminary observations on the prophylaxis of pyelonephritis during pregnancy by administration of sulfamethoxypyridazine. In the course of observations on pyelonephritis and on asymptomatic bacteriuria being made in our laboratory by Edward H. Kass,[5] he and an assistant, Joanne Norton, are carrying out a controlled study to determine the possible value of sulfamethoxypyridazine in the prevention of acute pyelonephritis in pregnant women found to have asymptomatic bacteriuria. A progress report of this study may be of interest.

The present study was performed for two reasons: (1) to determine the prognostic significance of asymptomatic bacteriuria during pregnancy, and (2) to

determine the clinical results of treatment of such bacteriurias with the long-acting sulfonamide, sulfamethoxypyridazine. Eleven prenatal patients with bacterial counts greater than 10^5 per ml. of freshly voided urine were studied. These were alternated between treatment with sulfamethoxypyridazine, 0.5 gm. per day, and administration of 1 capsule daily of a placebo. The patients were followed for 1 to 4 mo. from the time the bacteriuria was first detected until the time of delivery. Treatment was given throughout this period, but was terminated occasionally during the week or two before the estimated date of confinement.

One patient did not return for follow-up, and is therefore not included. Of the remaining 10, 6 received sulfamethoxypyridazine and 4 the placebo. Five of the 6 sulfamethoxypyridazine-treated patients, all of whom had *E. coli* in the urine, were found to be free of coliform rods in their urine within the week after treatment was started. One patient who took her pills irregularly did not respond; her organisms persisted even at a time when there were low but measurable levels of the sulfonamide in the blood and urine. After about 2 months the organisms still persisted, so this patient was treated with tetracycline, and the organisms were eliminated. None of the 6 patients treated with sulfamethoxypyridazine evidenced symptoms of urinary tract infections. No other untoward reactions were noted, and no azotemia or leukopenia appeared. Blood levels of free sulfonamides were maintained between 1 and 10 mg. per 100 ml., and urine levels were approximately 2 to 3 times as high, but there was considerable fluctuation from patient to patient.

Of the 4 placebo-treated patients, 3 developed clinical evidence of active infection of the urinary tract: 1 during the second trimester, 1 during the ninth month, and the third immediately after delivery. In all cases the organism in the urine was the same as the one that had been found repeatedly during the previous period of prenatal observation. In each instance there was prompt symptomatic relief and clearing of the bacteriuria upon administration of antimicrobial treatment.

It is tentatively concluded from this study (1) that asymptomatic bacteriuria may be associated with the later development of clinical evidence of pyelonephritis in pregnant women; (2) that preliminary evidence indicates that antibacterial treatment may be of value in preventing the appearance of some cases of pyelonephritis of pregnancy; and (3) that sulfamethoxypyridazine is a sufficiently simple and effective means of achieving such prophylaxis to warrant further study.

Untoward effects. No untoward effects were observed from either drug in any of the patients in the present studies. Crystalluria was not noted in any of the freshly voided specimens, although some of the sulfachloropyridazine crystallized out from the more concentrated specimens of urine from patients receiving sulfachloropyridazine.

Summary and Conclusions

Some additional observations on the levels of sulfamethoxypyridazine (Kynex) in blood and urine have been presented, and comparisons have been made with the related sulfonamide, sulfachloropyridazine (Ba-10370).

After single oral doses, the methoxy derivative has been shown to have a half life in plasma about 5 times as long as that of the chloro derivative. The time required to excrete one half of an oral dose or one half of the total amount that can be recovered in the urine was about 7 times as long for sulfamethoxypyridazine as for sulfachloropyridazine.

The concentrations obtained in the blood from 0.5 gm. of sulfamethoxypyridazine every 12 hr. showed a tendency to cumulate during a period of 7 days, but there was little fluctuation between doses. Levels of the same order of magnitude were obtained in the blood during the administration of 1 gm. of sulfachloropyridazine every 6 hr., but there were greater fluctuations in these blood levels between the latter doses.

Concentrations in the urine were considerably higher, and a somewhat smaller proportion was observed in the conjugated form during administration of 4 gm. daily of sulfachloropyridazine as compared with those obtained on 1 gm. daily of sulfamethoxypyridazine.

Preliminary controlled observations in pregnant women suggested that the finding of asymptomatic bacteriuria may be associated with the later development of acute pyelonephritis. Such acute episodes may be prevented by continuous administration of small doses of sulfamethoxypyridazine in the order of 0.5 gm. daily.

Both drugs were well tolerated in the doses used, and no untoward effects have been observed in the present study.

The differences in the absorption and excretion of these two sulfapyridazines suggest differences in their potential areas of usefulness. The high blood levels and slow urinary excretion of sulfamethoxypyridazine may permit the use of relatively low and infrequent doses, suggesting that this drug should have potential uses when prolonged treatment or prophylaxis is required and when sustained high levels in blood and tissue fluids are important. Sulfamethoxypyridazine may be useful also in maintaining prolonged suppression of infection. Also, this compound should prove to be more economical and more acceptable for prolonged treatment because of the smaller and relatively infrequent doses that are required. Sulfachloropyridazine, because of its rapid excretion and attainment of high concentrations in the urine, may be useful in urinary tract infections. However, the relatively large doses required to sustain satisfactory levels in the blood indicate that crystalluria and resulting irritation of the urinary tract may be potential drawbacks when such large doses are used.

Acknowledgments

The chemical determinations in this work were carried out by Ellen J. Doyle and Mary I. Kendrick.

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USE OF SULFAMETHOXYPYRIDAZINE IN THE PREVENTION OF STREPTOCOCCAL INFECTIONS IN RHEUMATIC PATIENTS

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It has been found that in human subjects the administration of sulfamethoxypyridazine* (3-sulfonamido-6-methoxypyridazine) is followed by high serum concentrations for 24 hours and by moderate concentrations for over 100 hours.[1, 2] Moreover, the antibacterial activity has been said to be of the same order of magnitude as that of sulfadiazine, and the toxicity has been reported to be low in animals and, thus far, in human patients. For this reason sulfamethoxypyridazine promises special usefulness in situations where sulfonamides have been of value, particularly where they have been used over prolonged periods of time. One such situation has been the use of these drugs for the prevention of implantation of group-A *Streptococcus pyogenes* in patients who have previously had rheumatic fever, in an attempt to reduce the number of recurrences and minimize progressive damage.[3-5] However, the currently recommended use of 1 gm. of sulfadiazine daily for children weighing over 60 lb., and one half as much for the smaller child[6] has suffered from a serious lack of patient cooperation. If sulfamethoxypyridazine could be used for this type of prophylaxis, with less frequent administration required, greater patient cooperation and reliability might be achieved. Moreover, the total required dosage would be less and, therefore, ultimately more economical. This report presents preliminary results of an attempt to develop a satisfactory method by which sulfamethoxypyridazine could be used conveniently and economically in the prophylaxis of streptococcal infections in rheumatic subjects.

Method

Two groups of patients were studied. Approximately half of the patients in Herrick House, the rheumatic fever convalescent home at Bartlett, Ill., were treated for the past 18 months with the new compound; the others were treated either with sulfadiazine or penicillin. In this group, serial serum sulfonamide concentrations were determined in 27 patients at weekly intervals extending in different individuals for from 3 to 21 weeks. In 19 of these the observation period was 7 or more weeks. Another 12 patients also received the drug. A total of 39 patients were observed over a period of 384 patient weeks in the institution. Weekly throat cultures were made for group-A *Streptococcus pyogenes*.

In the second group, ten outpatients were treated in an outpatient department by Marienfeld. In these 10 patients there were 213 patient weeks of observation. The patients were seen at monthly intervals and had serum sulfonamide determinations and throat cultures at each observation. In addition,

* Generously provided by Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y., under the registered name Kynex.

antistreptolysin "O" antibodies were determined whenever there was a question concerning possible infection during the preceding month.

In each of these groups, those on sulfamethoxypyridazine received a single dose of 30 mg. per kg. once a week. Blood specimens were obtained routinely for the serum sulfonamide determination just prior to the next weekly dose, and thus represented the lowest level reached in the serum between doses. In 6 patients at Herrick House who were on the standard sulfadiazine regimen, serum concentrations were determined at 24 hours, or the low point immediately preceding daily medication. In addition, on 2 patients receiving sulfamethoxypyridazine but with consistently less than 1 mg. per 100 ml. at the low point, determinations were made in the middle of the week as well as at the end for several weeks. Following this, the dose was increased to 30 mg. per kg. twice weekly, and the serum concentrations were again determined at 7-day intervals just prior to one of the doses of medication. The concentrations of free and total sulfonamide calculated as sulfamethoxypyridazine were determined by the method of Bratton and Marshall.[7]

Serum-Free and Conjugated Drug Concentrations

In TABLE 1 are listed the mean serum sulfonamide concentrations (presented graphically in FIGURE 1) in the serum of 19 patients who had 7 or more weekly

TABLE 1
PLASMA SULFONAMIDE CONCENTRATION 1 WEEK AFTER ADMINISTRATION OF 30 MG./KG. OF SULFAMETHOXYPYRIDAZINE

Patient	No. of weekly determinations	Sulfanomide concentration (mg./100 ml. ± S. D.)		Weekly determinations below 1 mg./100 ml.	
		Free	Conjugated	Number	Per cent
1	13	5.0 ± 0.98	0.47 ± 0.12	0	0
2	7	2.5 ± 1.0	0.34 ± 32	1	14.3
3	21	2.4 ± 0.58	0.32 ± 0.27	0	0
4	8	2.4 ± 0.54	0.47 ± 0.20	0	0
5	17	2.3 ± 0.62	0.41 ± 0.29	0	0
6	8	2.2 ± 1.0	0.83 ± 0.32	0	0
7	8	2.2 ± 0.56	0.65 ± 0.43	0	0
8	12	2.1 ± 1.5	0.19 ± 0.13	0	0
9	19	2.1 ± 0.57	0.54 ± 0.46	0	0
10	9	2.0 ± 0.97	0.31 ± 0.21	2	22.3
11	10	1.6 ± 0.31	0.24 ± 0.17	0	0
12	7	1.3 ± 0.23	0.16 ± 0.12	0	0
13	10	1.1 ± 0.26	0.10 ± 0.10	3	30.0
14	10	1.0 ± 0.63	0.12 ± 0.15	4	40.0
15	11	0.90 ± 0.10	0.24 ± 0.07	9	81.7
16	9	0.83 ± 0.17	0.18 ± 0.10	7	77.8
17	15	0.71 ± 0.62	0.07 ± 0.15	11	73.3
18	12	0.70 ± 0.59	0.10 ± 0.09	10	83.4
19	7	0.30 ± 0.06	0.01 ± 0.03	7	100.0
20–27*	34	1.8 ± 1.5	0.36 ± 0.36	13	38.3
Total	247	1.83 ± 1.4	0.31 ± 0.30	67	27.1
Sulfadiazine control†	6	0.65 ± 0.43	0.20 ± 0.30	4	66.7
Outpatients	23	1.50 ± 1.47	0.34 ± 0.51	9	39.1

* Three to 6 observations at weekly intervals per patient.
† Serum sample obtained 24 hours after a daily 1-gm. dose.

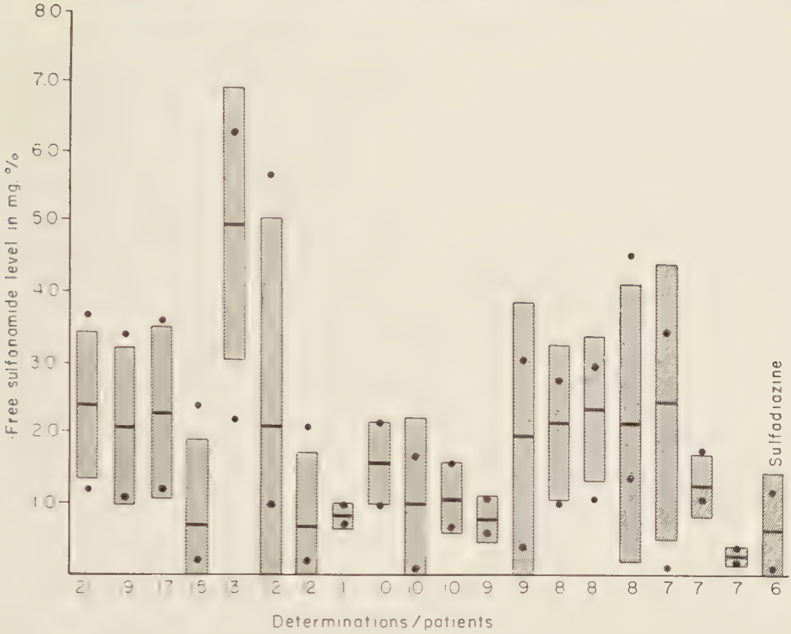

FIGURE 1. Mean of repeated determinations of serum sulfamethoxypyridazine concentration 1 week after the administration of the drug. The patient was treated with 30 mg. per kg. once each week. Ranges of plus or minus 1 standard deviation and maximum and minimum values are indicated. The sulfadiazine column represents the mean values of sulfadiazine serum concentration from 6 patients. Samples were obtained 24 hours after a daily 1 gm. dose.

determinations performed. Considered together are 8 other patients with fewer observations per patient treated in the convalescent home, 6 patients who received sulfadiazine, and 10 patients treated in the outpatient clinic. It can be seen that the mean of the lowest readings of the patients treated with the new pyridazine in the hospital was significantly higher (t = 2.06) than the mean for those patients who received sulfadiazine. Other than this, there are no significant differences among the mean values for the total group in the hospital, for the total for the outpatients, for the total for the patients who had only a few determinations per patient (20 to 27), and for the total for the patients treated with sulfadiazine. On the other hand, as can be seen in FIGURE 2, the mean value for the free sulfonamide concentrations among different patients on whom 7 or more tests were done are frequently significantly different. This illustrates that there was considerable consistency among the serum concentrations when they were measured at the same time each week and when the concentrations were at a minimal level. Likewise, most of the concentrations of less than 1 mg. per 100 ml. were found repeatedly in the same patients, while others had no determinations that low. There was a significantly higher percentage of patients in the sulfadiazine group who had serum concentrations below this level than in the total group in the home (Chi square 4.45).

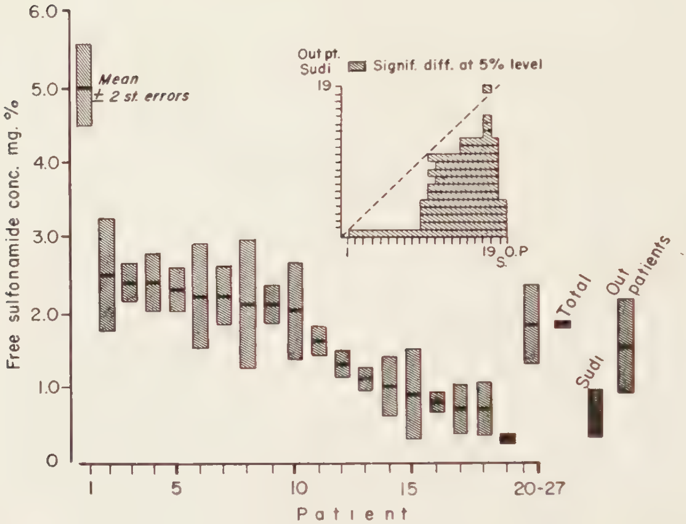

FIGURE 2. Mean of repeated determination of serum sulfamethoxypyridazine concentrations 1 week after the administration of a weekly dose of 30 mg. per kg. The range represents plus or minus 2 standard errors. The sulfadiazine column represents the mean values of sulfadiazine serum concentration from 6 patients. The samples were obtained 24 hours after a daily 1-gm. dose. The insert indicates those patients whose determinations (shaded area) were significantly different from those for the patient represented by the horizontal bar.

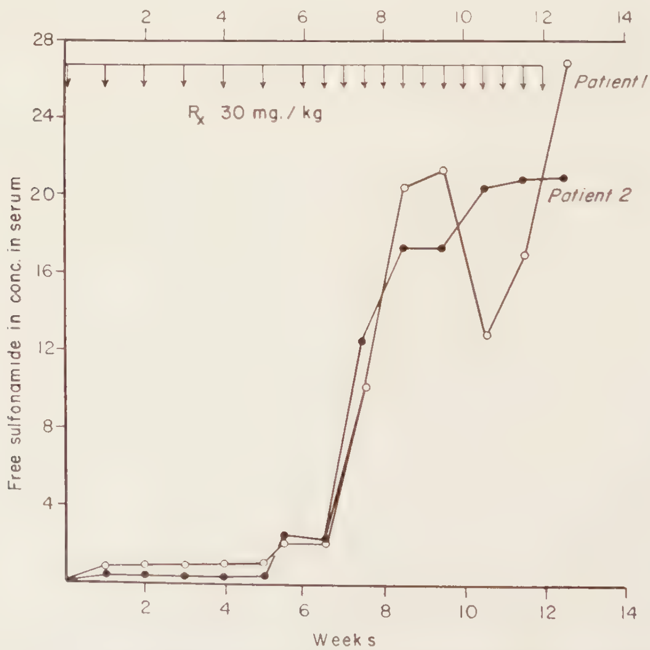

FIGURE 3. Weekly sulfamethoxypyridazine serum concentrations in 2 patients receiving 30 mg. per kg. once a week and then twice a week.

In FIGURE 3 are illustrated the data from 2 patients who were studied in detail because they were in the group that consistently had serum concentrations below 1 mg. per 100 ml. while they were receiving 30 mg. per kg. once a week. Sera obtained on the fourth day after the dose in each patient contained approximately 2 mg. 100 ml. The same dose was then administered twice a week, and serum concentrations were determined once a week just prior to one of the doses. As can be seen, there was a rapid increase in the concentration to a plateau 8 to 10 times as high as the concentrations determined immediately preceding a weekly dose.

FIGURE 4 illustrates the ratio of free drug to conjugated drug in the sera of patients with more than 6 determinations of each. It can be seen that the mean ratio varied from patient to patient and that the value was more constant in some than in others. However, it was constant enough to demonstrate that the mean ratios for many patients were significantly different from those of other patients. In spite of the fact that the percentage conjugated varied with different individuals, the absolute concentration was corrclated to the absolute concentrations of the free drug. Thus, for the 247 determinations

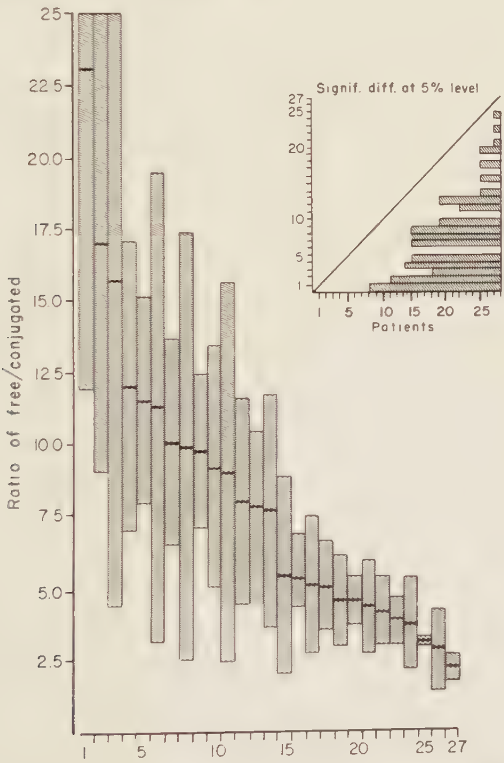

FIGURE 4. Mean ratio of free to conjugated serum sulfamethoxypyridazine concentrations for different patients plus or minus 2 standard errors. The insert indicates those patients whose determinations (shaded area) were significantly different from those for the patient represented by the horizontal bar.

performed on patients in the hospital, the mean free level was 1.83 mg./100 ml., and the conjugated level was 0.31 mg./100 ml., with the coefficient of correlation $r = 0.470$. The t value for this r was 8.4, a highly significant result. However, the consistency of the percentage conjugated among individuals and the differences between them were not dependent on the total concentration, as can be seen by inspection of TABLE 1. Moreover, in the 2 patients in FIGURE 3, when the serum concentration was higher at the earlier period or raised by more frequent drug administration, the percentage conjugated did not increase proportionately.

It was observed also that while there was no consistent trend in individual patients as to the fraction conjugated, with the passage of time there was, in the total data, some decrease in the proportion of free drug in patients treated for more than 10 weeks. Thus, among 208 readings made on patients in the first 10 weeks of treatment, the mean of the ratios of free to bound drug was 9.1 to 1, with a standard deviation of 10, whereas for 39 readings from the second 2 weeks, the mean of the ratios was 5.1 to 1, with a standard deviation of 3.1. This difference between the means is significant at the 5 per cent level, since t equals 2.41.

Bacteriological Results

Among the entire group of patients treated at Herrick House, there were 384 patient weeks of observation, with serial throat cultures taken each week. Not one of these cultures was positive for group-A hemolytic *Streptococcus*. It is also true, however, that cultures similarly performed on all the other patients receiving sulfadiazine or penicillin were also negative, as were almost all of the cultures on the employees of the institution, who were not receiving antibiotics or sulfonamides. Thus, these patients were in a low-risk situation.

On the other hand, among the 10 patients followed for 213 patient weeks at monthly intervals in the outpatient department, the risk was greater. Beta-hemolytic streptococci, group unknown, were isolated a total of 4 times from 3 of these patients. The 2 patients with single isolates had them on the same day, 13 weeks after the start of treatment, and have not had them since that time. The third patient had a positive culture on the ninth and thirteenth week, at which time he was given penicillin and was then continued on the sulfamethoxypyridazine. The culture did not become positive again. From the start of treatment, this patient and 1 of the 2 patients with a single positive culture showed a consistent fall in the antistreptolysin titer. The occurrence of the positive culture did not alter the rate of the fall in this antibody titer; there was no elevation of the sedimentation rate or of the C-reactive proteins, and no evidence to suggest an activation of rheumatic fever in any subject with positive cultures. The occurrence of positive cultures did not correlate with the serum concentrations, since several of the highest levels were in patients with positive cultures.

The drug was well tolerated. Several patients had transitory headaches shortly after the start of treatment, but this effect disappeared following the later doses. There were no hematological abnormalities, and it was not necessary to discontinue the drug in any patient because of toxicity.

Discussion

Since it has become apparent that repeated acute attacks of rheumatic fever are caused by group-A streptococci, means of preventing such infections in patients who have had rheumatic fever have been sought. Several agents have been shown to be effective, but daily sulfadiazine or penicillin, either as benzathine salt by injection or as potassium salt orally administered are currently recommended.[6] It has been felt, however, that sulfamethoxypyridazine might be successful in preventing such infections when given less frequently than daily, as with other oral therapy, and in a smaller dose than is necessary with sulfadiazine.

The results reported suggest that the drug has properties that justify such hopes. The drug was capable of persisting in the serum and presumably in the tissues for several days after a single dose. Moreover, the concentrations obtained from a single dose were so consistent for each patient, that, when a satisfactory dose had been chosen, it could be counted upon to give adequate coverage. At the present time, however, it is not possible to predict the serum concentration necessary for optimal protection. From the results, it would seem possible that 30 mg. per kg. twice a week is more than is needed, even in patients with consistently low concentrations one week after a single dose. Since 30 mg. per kg. once a week maintained generally higher concentrations at the lowest level than the conventional sulfadiazine regimen, it was felt that a trial at this dose level was warranted. It is obvious, however, that a relatively low concentration over a period of days may be quite different therapeutically than daily transitory concentrations of the same level. In view of the freedom from infections in the hospital among these patients, the efficacy of this regimen would not be demonstrated conclusively.

The results obtained in the outpatient department are not conclusive as yet. It is clear that there has not been sufficient infection to cause either a rise in the antistreptolysin titer or a recurrence of rheumatic fever. Among the several positive cultures, however, there may have been some group-A organisms. Unfortunately, these isolates could not be grouped.

One of the problems that has occurred with all methods of oral prophylaxis has been lack of cooperation. It was felt that a drug that could be given as infrequently as once a week would encourage patient reception. The fact that the positive cultures occurred in patients who had demonstrable serum concentrations, and also that the distribution of concentrations was almost identical with those obtained among the patients known to be taking the drug, suggested increased cooperation by the patients.

The concentrations of the conjugated drug suggested a somewhat different limitation for each patient on the amount that can be conjugated, and implied that the maximum amount may increase slowly when the drug is used over a prolonged period.

Summary

(1) Sulfamethoxypyridazine was given in a dose of 30 mg. per kg. per week to 39 patients in a rheumatic fever home. All of these patients remained free

of streptococci in their throats. Serum obtained on 27 patients at the end of 7 days just prior to the next dose averaged 1.8 mg. per 100 ml. Less than one third of such sera contained below 1 mg. per 100 ml. These results were superior to those obtained in 6 patients receiving 1 gm. of sulfadiazine per day.

(2) Two patients who had very low serum concentrations received 30 mg. per kg. twice a week. The serum concentrations in these patients was approximately 20 mg. per 100 ml.

(3) The serum-conjugated sulfonamide concentration was correlated roughly to the height of the free drug concentration. However, when the serum concentration was increased by doubling the dose, the percentage conjugated was markedly lower. The percentage of conjugation varied between patients more than in the individual patient. It increased slightly after ten weeks of treatment.

(4) The drug has had a preliminary trial in ten patients in a rheumatic fever clinic with satisfactory results.

Acknowledgments

The authors thank the personnel of Herrick House for their kind cooperation, and Betty Ross Connor for her technical assistance.

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SULFAMETHOXYPYRIDAZINE: PHARMACOLOGICAL OBSERVATIONS AND CLINICAL USE IN THE TREATMENT OF URINARY TRACT INFECTIONS

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Several potentially favorable pharmacological properties of sulfamethoxypyridazine (3-sulfanilamido-6-methoxypyridazine) were suggested by preliminary experimental data.[1] Among these were a slow rate of excretion, a low degree of acetylation, absorption from the gastrointestinal tract, greater solubility in acid media than other sulfonamides, and antibacterial activity comparable to that of sulfadiazine.

Our initial investigations confirmed these pharmacological observations in humans. Three patients who weighed 60 to 75 kg. were given a single oral dose of 2.5, 5.0, or 7.5 gm. of sulfamethoxypyridazine. The dose was tolerated well. Blood was collected 4 hr. after the dose and each 24 hr. thereafter up to 8 days. All urine was collected as individual specimens during the first 48 hr., then as pools of urine for each 24-hr. period.

Blood Concentrations

The blood concentrations of free and total sulfonamide, as determined by the Bratton-Marshall reaction,[2] are given in TABLE 1. The level 4 hr. after administration was comparable regardless of the size of the dose given, and the peak concentration was attained between 4 and 24 hr. When the 2.5-gm. dose was given, the blood concentration declined approximately 50 per cent each 24 hr. A single dose of 5.0 or 7.5 gm. sustained the blood levels, which showed a gradual decline of 10 to 30 per cent each 24 hr. An appreciable level was still demonstrable 8 days after a dose of 7.5 gm. Thus, the effect of increasing the amount of a dose within the range observed was one of sustaining rather than markedly elevating the blood concentration. The serum concentration was found to be the same as that for whole blood corrected for the hematocrit. The proportion of drug circulating in the conjugated, presumably acetylated, form varied among individuals, but was usually less than 10 per cent of the total.

Urine Concentrations

The concentration of sulfonamide in the urine was inversely proportional to the urine volume, and thus the excretion was relatively independent of the urine flow. The patient who received a single oral dose of 2.5 gm. excreted 795 mg. during the first 24 hr. (TABLE 2). The total amount of drug excreted by each of the patients 48 hr. after the dose varied less than 100 mg., regardless of the size of the initial dose. When 5.0 or 7.5 gm. had been given, the high rate of excretion, 300 to 600 mg. per day, continued for several days. During the period of observation, approximately one half of the administered drug was recovered in the urine. Continued studies with the first patient,

TABLE 1

Time after dose	Blood concentration (mg. %) of sulfamethoxypyridazine, single oral dose					
	Dose (gm.)					
	2.5		5.0		7.5	
	Free	Total	Free	Total	Free	Total
4 Hours	11.4	12.4	10.2	10.4	12.5	13.8
24 Hours	6.8	8.0	11.5	11.5	10.0	12.7
48 Hours	3.6	4.2	10.4	11.0	7.2	10.0
3 Days			7.6	7.8	4.2	6.7
4 Days			5.5	5.7		
6 Days					1.1	2.0
8 Days					1.0	1.7

(Blood: serum $= \frac{1 - \text{Hct}}{1} \pm 0.5$ mg. %.)

TABLE 2

Time after dose	Urinary excretion of sulfamethoxypyridazine					
	Dose (gm.)					
	2.5		5.0		7.5	
	Mg. recovered	Per cent of dose	Mg. recovered	Per cent of dose	Mg. recovered	Per cent of dose
4 Hours	52	2				
24 Hours	795	32	320	6	719	11
48 Hours	1331	54	1274	26	1243	17
3 Days			1899	38	1521	20
4 Days			2343	47	2154	29
5 Days			2678	53	2543	34
6 Days					2734	37
7 Days					2914	39
8 Days					3085	40
Free/acetyl	1:1		10:1		1:2	

however, netted 70 per cent recovery of 7.5 gm. that had been administered in 3 doses during 1 week. The chemical form in which the drug was excreted showed marked individual variation. Two of the patients excreted one half and two thirds of the total as acetylated drug, whereas 90 per cent of that excreted by the other patient was free sulfonamide. The former pattern was more characteristic of other patients although, with repeated administration, a larger proportion was excreted as free drug.

Renal Clearance and Excretion

Because of the relatively constant plasma levels, it was possible to calculate renal clearances and the rate of urinary excretion. These data for one patient are plotted in FIGURE 1. The renal clearance of free drug at various levels of

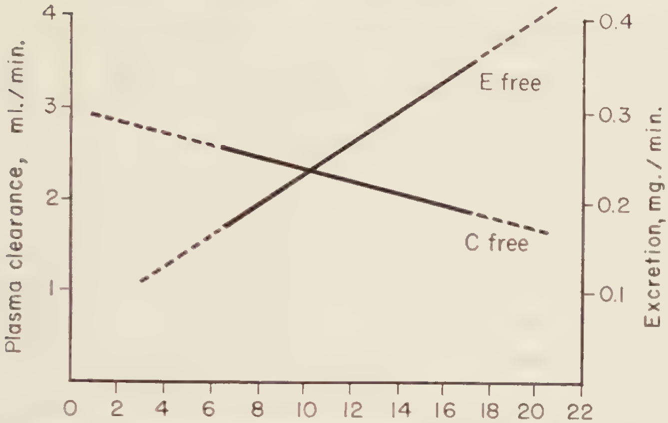

FIGURE 1. Renal clearance and excretion of sulfamethoxypyridazine.

plasma concentration was relatively constant between 2 and 3 ml./min., but there was a linear decline in the rate of clearance at increasing concentrations, which suggested a threshold phenomenon. The rate of excretion had the opposite relationship, and the milligrams of free drug per minute increased in linear proportion to the plasma concentration. The rate of renal clearance of free sulfonamide, however, was found to be an individual variable. Rates of 0.9 to 5.6 ml. min. were observed. Readministration of the drug as well as the plasma concentration influenced the rate of renal clearance. As much as 100-per cent increase was observed at the same plasma concentration upon successive days of treatment. A similar increase occurred in the proportion of drug excreted as free sulfonamide.

The renal clearance of conjugated, presumably acetylated, sulfonamide was 4 to 10 times higher than that for sulfamethoxypyridazine in the same patient and ranged from 6 to 24 ml. min. Similar results have been obtained by other workers.[3-5]

Maintenance Therapy

Among the patients given sulfamethoxypyridazine for therapeutic purposes, the majority received 2.5 gm. initially and 0.5 gm. each day for as long as 250 days. The mean duration of treatment was 30 days. Two thirds of the patients were treated for 3 to 4 weeks. The serum concentrations in 125 random specimens on a 0.5 gm. daily maintenance dose are shown in FIGURE 2. Although there was considerable variation, the majority of the specimens contained between 5 and 15 mg. per cent of free sulfonamide, with a mean concentration of 10.3 mg. per cent. No significant accumulation of drug was observed at this level of maintenance.

Urine specimens were obtained simultaneously with the blood from patients receiving maintenance therapy. The frequency distribution of 104 specimens according to the concentrations of free sulfonamide in the urine is shown in FIGURE 3. In 94 per cent of the samples the concentration exceeded 5 mg. per

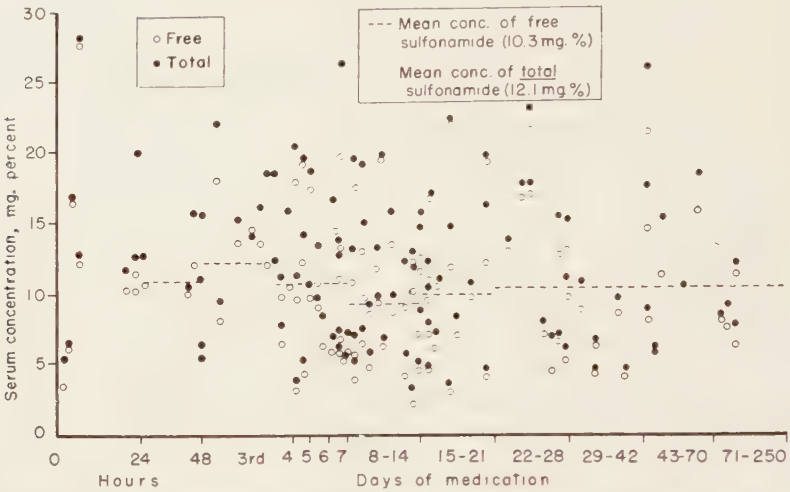

FIGURE 2. Serum concentrations in 125 random specimens on a 0.5 gm. daily maintenance dose.

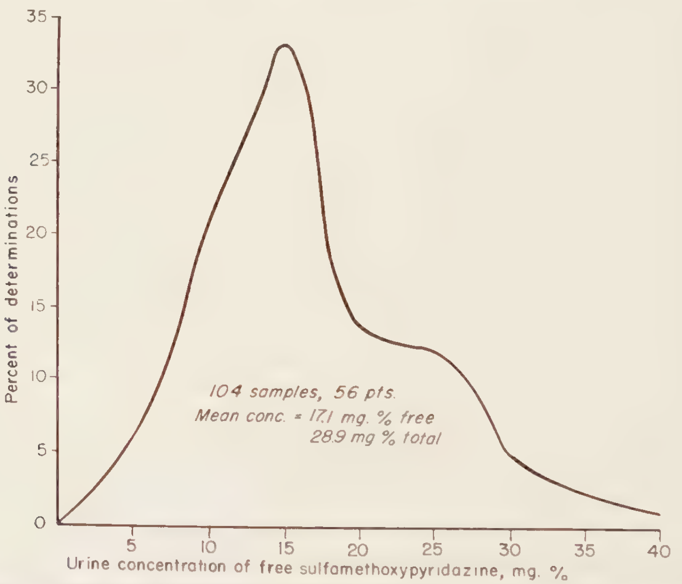

FIGURE 3. Frequency distribution of 104 specimens according to the concentrations of free sulfonamide in the urine.

cent; the mean was 17.1 mg. per cent, and the mean concentration of total sulfonamide was 28.9 mg. per cent. Thus, the ratio of free to conjugated drug in the urine was 3:2. During the first week the concentration in the urine was 25 to 50 per cent higher owing to the initial dose of 2.5 gm., but from the 2nd to the 35th week more than 90 per cent of the specimens contained a concen-

TABLE 3

ANTIMICROBIAL EFFECT OF SULFAMETHOXYPYRIDAZINE UPON INFECTED URINE

Species	Number of strains				
	Eradicated	Suppressed	Persisted	Emerged	Total
Gram-negative					
Escherichia coli	16	2	9	1	28
Coliform	2	2	1	5	10
A. aerogenes	2	—	6	2	10
Proteus species	4	3	6	1	14
Pseudomonas	—	1	2	3	6
Salmonella cerro	—	—	1	—	1
Subtotal	24	8	25	12	69
Gram positive					
Streptococcus, Gr. D	4	2	6	8	20
Streptococcus, Gr. B	1	0	0	2	3
Streptococcus, α, γ	1	1	0	3	5
Micrococcus, Coag. −	1	2	2	5	10
M. pyogenes, Coag. +	—	—	—	1	1
Subtotal	7	5	8	19	39

tration of free sulfonamide greater than 5 mg. per cent, with a mean of 12.9 mg. per cent. The mean concentration of total sulfonamide during this period was 19 mg. per cent. At these levels sulfonamide crystalluria was observed in only 1 patient.

In Vivo Antibacterial Activity

Among the 56 patients treated, 39 had complete bacteriological studies of the urine before, during, and at least 3 to 4 weeks after the completion of treatment, which enabled evaluation of the *in vivo* antimicrobial effects of sulfamethoxypyridazine upon the bacterial strains in the urine. One half of the patients suffered from chronic pyelonephritis; 70 per cent of this group had contributing urologic or metabolic disease, and many had failed or relapsed after previous treatment with antibiotics. The data are shown in TABLE 3. Sixty-nine strains of Gram-negative bacteria were encountered. Among 57 that were isolated before treatment, 24 or 42 per cent were eradicated from subsequent cultures, and 8 or 14 per cent disappeared transiently during the period of drug administration. Thus, the drug was effective against 56 per cent of the Gram-negative strains. Twenty-five strains or 44 per cent were not influenced by treatment. *Escherichia coli*, *Escherichia freundi*, paracolon bacillus, *Aerobacter aerogenes*, *Proteus mirabilis*, and *Proteus rettgeri* were among the species effectively treated. Only among strains of *E. coli* and the closely related coliform species, however, were more than one half of the strains inhibited. Even among the coliform variants, new strains appeared more often than pretreatment strains were cleared, whereas strains of *E. coli* were effectively and sometimes dramatically cleared from the urine in 60 per cent of the

cases, and the emergence of new strains was rare. Sixty per cent of the strains among the genera of *Aerobacter*, *Pseudomonas*, *Proteus*, and *Salmonella* showed no response, and the number of new strains that emerged during treatment was equal to the number of pretreatment strains cleared.

Thirty-nine strains of Gram-positive microorganisms were isolated among the genera of *Streptococcus* and *Micrococcus*. Nearly one half of these emerged while the patients were receiving treatment; this often represented a significant change in the bacterial flora of the urine without clinical improvement. One third of the pretreatment strains were eradicated, however.

On a clinical basis, patients with acute symptoms and recent infections showed a more favorable response to treatment than those with chronic urinary tract infection. Seventy-five per cent of the former had a favorable clinical response and improvement in the urine sediment, whereas 30 per cent of the latter obtained similar benefit from treatment.

Adverse Reactions

Among the 56 patients who received one or more courses of treatment with sulfamethoxypyridazine, 5 (8.9 per cent) had unfavorable reactions of sufficient severity to warrant the discontinuation of the drug. The nature of these reactions is shown in TABLE 4. Two additional patients complained of severe headache, and another had a bout of marked dizziness following the initial dose of 2.5 gm. All of the patients in the latter group tolerated continued administration of the drug, and in 2 of the 3 patients the symptoms improved during the course of treatment.

Headache and rash were the most common reactions, but fever, abdominal pain, and dizziness also were observed. In all but one instance, the rash appeared after several days of treatment. The lesions were erythematous, maculopapular, and involved both the trunk and extremities; they were relatively non pruritic. In the other patient the rash appeared promptly but transiently after the first dose and caused some itching.

In the one patient with sulfonamide crystalluria there were no signs of renal toxicity. Leukopenia was noted in only one instance, and it almost certainly was a manifestation of the primary illness, Hodgkin's disease. No other

Table 4

Adverse Reactions* Among 56 Patients Receiving Sulfamethoxypyridazine

Symptom	No. of persons	Per cent occurrence
Headache	4	7.2
Rash	4	7.2
Fever	2	3.6
Stomach-ache, vomiting	2	3.6
Dizziness	1	1.8
	5	8.9

* Required discontinuation of treatment.

TABLE 5

READMINISTRATION OF SULFAMETHOXYPYRIDAZINE TO PATIENTS WHO SHOWED AN ADVERSE REACTION

Patient	E. I.				F. B. S.				I. J. D.				R. W.		G. R.	
Course	1	2	3	4	1	2	3	4	1	2	3	4	1	2	1	2
Dose, gm./day	2.5	0.5	0.5	0.5	1.0	0.5	0.5	0.5	0.25	0.25	0.25	0.25	0.5	0.5	0.5	0.5
Symptom																
Headache	×	×	0	×	0	0	0	0	0	×	×	×	×	×	×	×
Rash	0	0	0	0	×	0	0	0	0	×	0	0	×	×	×	×
Fever	0	0	0	0	0	0	0	0	0	0	×	×	0	0	×	×
Stomach-ache, vomiting	0	0	0	0	0	×	0	0	0	0	×	×	0	0	0	×
Dizziness	×	×	0	0	0	0	0	0	0	0	0	0	0	0	0	×

blood dyscrasias were found, and no other signs of chronic toxicity were observed.

All of the five patients who had adverse reactions were retested by the administration of another dose or course of treatment after the manifestations of hypersensitivity had subsided. The observations are presented in TABLE 5. In all but one of the patients the reaction occurred during the first course of treatment. One patient developed symptoms only during the second and subsequent courses. Upon readministration of the drug, all of the patients had some symptoms, but two of the five were able to complete a subsequent course or courses of treatment without appreciable drug toxicity.

One patient, G. R., had a prompt severe reaction to the readministration of 0.5 gm. Nausea and vomiting were followed by chills and fever. The blood pressure fell to shock levels and oliguria was present for 2 days. A generalized rash appeared approximately 6 hours after ingestion of the test dose. Periorbital edema and swelling of the hands occurred later.

Three patients not included in the present data were tested with sulfamethoxypyridazine after they developed overt hypersensitivity reactions to sulfisoxazole. None showed any untoward symptoms to doses of sulfamethoxypyridazine that produced a definite blood level.

Discussion and Summary

The pharmacological properties of sulfamethoxypyridazine are very favorable for clinical use. In our experience, a single daily dose produced plasma and urine concentrations that were generally accepted as adequate for antimicrobial activity. Long-term administration was well tolerated, and the crystallization of sulfonamide in the urine was not a problem. In urinary tract infections the agent exerted its greatest effect against strains of *E. coli* and was relatively less effective for inhibiting other gram-negative bacilli and gram-positive microorganisms. Significant adverse reactions similar in type and frequency to those caused by other sulfonamides were observed, but they produced no serious sequelae. Readministration of the drug to these patients, however, might be dangerous.

Acknowledgment

The authors gratefully acknowledge the assistance of Betty Connor, who performed the sulfamethoxypyridazine determinations.

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SULFAMETHOXYPYRIDAZINE, A LONG-ACTING SULFONAMIDE: SOME PRELIMINARY CLINICAL AND LABORATORY OBSERVATIONS IN INFANTS AND CHILDREN

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Introduction

Sulfonamides still deserve high-priority consideration in any discussion of antibacterial therapy, particularly in pediatrics. With the advent of new and potent antibiotics in the past ten years, the tendency has been to minimize the role of sulfonamides in current-day practice. Nevertheless, it is well to point out that these compounds still are important in the management of infections in children, although perhaps to a lesser extent in adults. Sulfonamides have the virtue of being rather inexpensive, of being easily administered orally, and of covering a relatively broad antibacterial spectral range.

A new antibacterial sulfonamide, 3-sulfonamido-6-methoxypyridazine, or sulfamethoxypyridazine (Kynex*), has recently been made available for clinical investigation. In animal experiments this compound has been shown to have a high solubility in urine, good absorption from the gastrointestinal tract, very slow urinary excretion, poor acetylation, good penetration into the spinal fluid, and an antibacterial activity equal to that of sulfadiazine.[1]

There have been several recent reports on the properties of sulfamethoxypyridazine when administered to adults in single 1- to 4-gm. doses.[2-4] In general, the observations have been comparable to those ascertained on animal experimentation. One observer, however, found comparatively poor diffusion across the blood-brain barrier in man as compared to animals.[2] Prior to the initiation of this study, no observations had yet been made regarding the clinical efficacy of the drug in adults or in the pediatric age group.

Methods and Materials

The purpose of the present investigation was as follows: (1) to determine the optimal dosage of sulfamethoxypyridazine in the pediatric age group, (2) to observe the range in blood levels when a constant dose was given at 24-hour intervals, (3) to compare its blood sulfa levels with those of other sulfonamides in equivalent dosage, (4) to determine the degree of diffusion across the blood-brain barrier, (5) to observe any untoward reactions, and (6) to accumulate some preliminary data regarding its efficacy in bacterial infections.

Sulfamethoxypyridazine and other sulfonamides were given on a dose-per-weight basis, as is customary in pediatric practice. Blood samples were obtained by micromethods. Both blood and spinal-fluid levels were determined promptly, employing a modified Bratton-Marshall technique in which

* The Kynex employed in this study was generously supplied by Stanton M. Hardy, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

only free sulfa was measured. Repeated blood counts, urinalyses, and clinical examinations were made during these studies.

Results

Optimal dosage. Single doses of sulfamethoxypyridazine of 100 mg., 50 mg., and 25 mg. per kg. of body weight were given to a total of 25 children varying in age from 3 months to 10 years and ranging in weight from 5.5 kg. to 25 kg. Free blood sulfa levels were determined at intervals of 2, 4, 8, 24, 48, and 72 hr.

The resulting free blood sulfa levels in mg. per cent were averaged and are presented in TABLES 1 through 3, and in FIGURE 1. As may be seen in TABLE 1, when a single 100 mg. per kg. dose of sulfamethoxypyridazine was administered, an adequate sulfa level was achieved in 2 hr., a peak level was achieved between 4 and 8 hr., and a gradual tapering of the blood sulfa concentration occurred thereafter. At the end of 48 hr. sulfa levels were still demonstrable in therapeutically significant concentration in the majority of children. At the end of 72 hr. the drug had either disappeared entirely or was present in suboptimal amounts. Thus, with a 100 mg. per kg. dose of sulfamethoxy-

TABLE 1
SULFAMETHOXYPYRIDAZINE, 100 MG./KG., SINGLE ORAL DOSE*

	Time					
	2 hr.	4 hr.	8 hr.	24 hr.	48 hr.	72 hr.
Average blood level (mg. %)........	9.3	16.7	17.3	13.7	7.9	3.4

* Ten patients, age 11 mo. to 7 years.

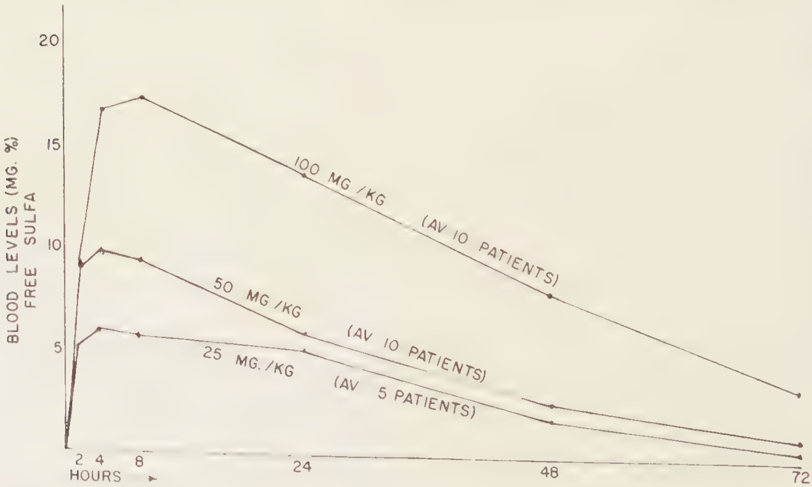

FIGURE 1. Comparison between sulfamethoxypyridazine blood levels (average) obtained after administration of a single dose of 25, 50, and 100 mg. kg.

TABLE 2
SULFAMETHOXYPYRIDAZINE, 50 MG./KG., SINGLE ORAL DOSE*

	Time					
	2 hr.	4 hr.	8 hr.	24 hr.	48 hr.	72 hr.
Average blood level (mg. %)	9.2	9.9	9.2	5.8	2.5	0.9

* Ten patients, age 4 mo. to 10 years.

TABLE 3
SULFAMETHOXYPYRIDAZINE, 25 MG./KG., SINGLE ORAL DOSE*

	Time					
	2 hr.	4 hr.	8 hr.	24 hr.	48 hr.	72 hr.
Average blood level (mg. %)	5.2	5.9	5.6	5.1	1.9	0.6

* Five patients, age 4 mo. to 10 years.

pyridazine, therapeutic levels were maintained for at least 48 hr. in the majority of children.

As may be seen in TABLE 2, with a single 50 mg. per kg. dose of sulfamethoxypyridazine a fairly adequate therapeutic blood concentration was achieved and maintained during the first 8 hr., followed by a gradual tapering off to a low therapeutic level at 24 hr. and a suboptimal level at 48 hr. and thereafter. From TABLE 3 it is apparent that a 25 mg. per kg. dose of the compound produced a constant, low therapeutic level for the first 24 hr., and only trace levels were present thereafter.

It is to be noted that the blood concentrations obtained with a single dose of 100 mg. per kg. of sulfamethoxypyridazine were approximately $1\frac{1}{2}$ to 2 times those obtained with 50 mg. per kg., and $3\frac{1}{2}$ to 4 times those obtained with 25 mg. per kg.

Daily fluctuations in sulfonamide levels. When clinical use of the drug was simulated, a single daily sulfamethoxypyridazine dose of 50 mg. per kg. was given for 5 consecutive days to 7 children ranging in age from 4 months to 10 years. Free blood sulfa levels were obtained at 4 and 24 hr. after each daily dose in an attempt to determine the highest and the lowest blood sulfa levels to be expected in therapy. The resulting average free blood sulfa levels in mg. per cent are presented in TABLE 4 and in FIGURE 2.

As may be seen from TABLE 4, the sulfonamide levels were maintained very adequately during the entire 5-day period. The levels ranged between 6.6 and 15.7 mg. per cent. These levels would constitute significant therapeutic blood concentrations for the majority of sulfa-susceptible infections.

Blood sulfonamide levels achieved with equivalent dosage by other sulfonamides. Sulfadiazine and sulfamerazine were each given to groups of 10 children in single oral doses of 50 mg. per kg. Blood sulfa levels were obtained at 2, 4, 8, 24, and 48 hr. The resulting levels of free blood sulfa were then compared

TABLE 4

SULFAMETHOXYPYRIDAZINE, 50 MG./KG., SINGLE ORAL DOSE DAILY*

Dose	Sulfa level (mg. %)	
	4 hours	24 hours
Day 1	9.5	6.6
Day 2	15.1	7.9
Day 3	13.4	8.1
Day 4	13.3	8.1
Day 5	15.7	9.7

* Seven patients, age 4 mo. to 10 years.

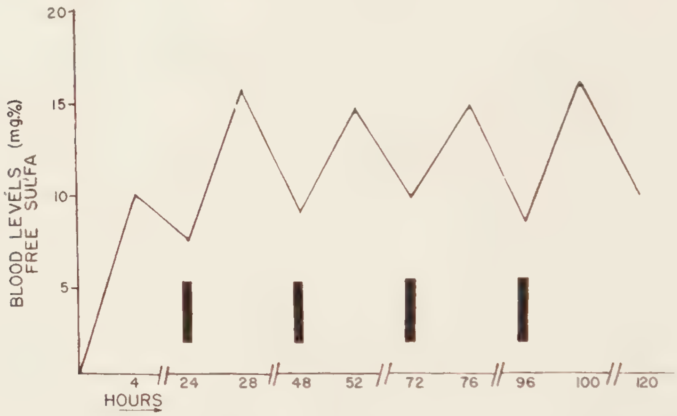

FIGURE 2. Sulfamethoxypyridazine blood levels obtained 4 and 24 hr. after 1 daily dose of 50 mg./kg. given for 5 consecutive days.

with the previously mentioned group of 10 children who had received a single dose of 50 mg. per kg. of sulfamethoxypyridazine in TABLE 2. The resulting average free blood sulfa levels obtained with sulfadiazine and sulfamerazine are given in TABLES 5 and 6.

As seen in TABLES 5 and 6, blood sulfa levels with both sulfadiazine and sulfamerazine were quite low during the first 24 hr. and were absent thereafter. By contrast (FIGURE 3), blood levels achieved with an equivalent dose of sulfamethoxypyridazine were 2 to 4 times as great during the first 24 hr., and there was still some concentration at 48 and 72 hr. This would indicate that sulfamethoxypyridazine substantially exceeds, both in magnitude and in duration, the blood levels that might be achieved with comparable doses of sulfadiazine and sulfamerazine.

Degree of diffusion of sulfamethoxypyridazine across the blood-brain barrier. A group of 7 infants and children with normal meninges were given a dose of 50 mg. per kg. of sulfamethoxypyridazine. Free sulfonamide blood and spinal fluid levels were obtained simultaneously at 4- to 8-hr. intervals thereafter. The resulting levels are presented in TABLE 7.

Maren *et al.*[1] had reported that sulfamethoxypyridazine penetrated the

TABLE 5

SULFADIAZINE, 50 MG./KG., SINGLE ORAL DOSE*

	Time				
	2 hr.	4 hr.	8 hr.	24 hr.	48 hr.
Average blood level (mg. %)........	3.5	4.8	4.0	2.0	0.0

* Ten patients, age 5 mo. to 10 years.

TABLE 6

SULFAMERAZINE, 50 MG./KG., SINGLE ORAL DOSE*

	Time				
	2 hr.	4 hr.	8 hr.	24 hr.	48 hr.
Average blood level (mg. %)	2.6	3.4	3.2	1.1	0.0

* Ten patients, age 6 mo. to 11 years.

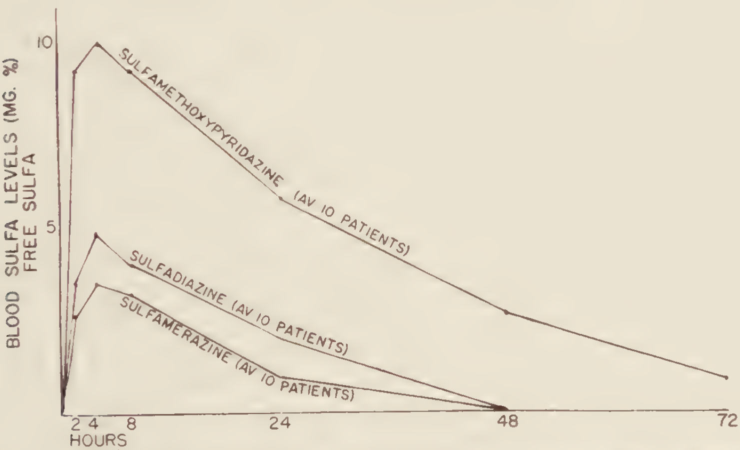

FIGURE 3. Comparison of blood sulfa levels of sulfadiazine, sulfamerazine, and sulfamethoxypyridazine after administration of a single dose of 50 mg./kg.

TABLE 7

Patient	Sulfa levels (mg. %).	
	Blood	Spinal fluid
1	5.8	0
2	13.0	1.2
3	4.4	0
4	9.0	0
5	13.0	0
6	11.6	1.2
7	10.4	1.2

blood-brain barrier well in animals with cerebrospinal sulfa levels approximately 55 to 60 per cent of the coexisting blood levels. Boger *et al.*[2] observed a much lower diffusion level in human adults. They have observed free sulfonamide in the cerebrospinal fluid ranging between 5 and 10 per cent of that observed in the plasma. As may be seen in TABLE 7, our results parallel those of Boger *et al.*, since in our study only 5.5 per cent of the coexisting blood sulfa level was demonstrable in the spinal fluid. It must be noted that 4 of the 7 children had no spinal fluid sulfa level, even though the coexisting blood level ranged between 4.4 and 13.0 mg. per cent. Thus, in the present series, in children with normal meninges, the diffusion of sulfamethoxypyridazine across the hematocephalic barrier was relatively low. One would expect some increase in diffusion in instances of inflamed meninges, but this point remains to be determined.

Incidence of side reactions to sulfamethoxypyridazine in children. Preliminary data were obtained on the 7 children who were given a daily single 50 mg. per kg. dose for 5 consecutive days. Hemograms and urines were obtained in each patient before therapy was begun and on every second day thereafter while on the drug. Blood urea nitrogen and thymol turbidity determinations were done prior to administration of the drug, and again on completion of therapy. Each patient was observed for untoward clinical manifestations such as rash, fever, and gastrointestinal disturbances. The incidence of side reactions was negligible in all 7 patients, with the exception of one clinically dehydrated patient who showed microscopic hematuria and a few sulfa crystals 4 days after sulfamethoxypyridazine was started. This child also ran a low-grade temperature up to 101° F. 3 days after therapy with sulfamethoxypyridazine was initiated. There was a question as to whether the latter represented drug fever or was a coincidental occurrence. In spite of hematuria, this child was maintained on the compound while she was being hydrated. Subsequently her urine cleared completely, and the fever subsided while on the drug. There was no discernible effect on either the blood urea nitrogen or thymol turbidity in any of these 7 children. Similarly, there was no evidence of any hematopoietic depression. No other clinical side reactions were observed.

In an additional 40 patients, blood counts and urines were performed during the course of drug administration, with no demonstrable resultant effect.

Out of a total of 49 children who received sulfamethoxypyridazine, two vomited the drug and were omitted from the study. A third child had loose stools. A fourth child developed a transient rash, possibly allergic in nature, within 2 days after discontinuation of the drug. In one instance a patient was given, by error, 50 mg. per kg. *every 6 hr.* instead of daily. Despite this, there was no side reaction and no abnormality of urine, blood count, or blood urea nitrogen.

Clinical evaluation. Thus far, a group of 14 children ranging in age from 2½ months to 12 years has been treated with sulfamethoxypyridazine alone in 50 mg. per kg. daily dosage for a variety of bacterial infections. The diseases treated, with a clinical and laboratory response designated as good, fair, or poor, are presented in TABLE 8.

TABLE 8
CLINICAL EVALUATION OF SULFAMETHOXYPYRIDAZINE

	Good	Fair	Poor
Upper respiratory infections	6		2
Bronchopneumonia	2		1
Shigella dysentery	1		
Cystitis	1		
Cellulitis	1		
Total	11		3

It was our general impression that, by and large, the drug was well tolerated in this small group of children. One needs little reminder, however, that observation of a new drug on a widespread scale clinically is the only reliable index of any potential toxicity and of therapeutic efficacy.

With the group of bacterial infections referred to in TABLE 8 the response to sulfamethoxypyridazine in a single daily dose appeared to approximate the results one might anticipate with sulfadiazine. However, it would be difficult to quantitate this impression clinically.

Conclusion

A group of 47 children ranging in age from 2 months to 12 years have received sulfamethoxypyridazine. The drug was taken readily and absorbed well, producing considerably higher and more prolonged free sulfa levels with a single daily dose than could be produced with equivalent dosage of sulfadiazine or sulfamerazine.

In children with normal meninges, there was relatively little diffusion (5.5 per cent) of the drug across the blood-brain barrier into the spinal fluid, although one might postulate that inflamed meninges might enhance such diffusion.

In calibrating the optimal dosage of sulfamethoxypyridazine in this series, it was felt that a single daily dose of 50 mg. per kg. would produce adequate free blood sulfa levels for therapy of the average mild to moderate sulfa-susceptible bacterial infection. In severe infections, however, it would seem advisable to employ a dose of 100 mg. per kg. daily during the acute phase of the disease.

Sulfamethoxypyridazine would appear to have an advantage in pediatric practice in producing a reasonably antibacterial therapeutic effect with a *single* oral dose of medication daily. The indications for use of the drug would seem to be the same as those for any other oral sulfonamide. One might anticipate that a single dose of sulfamethoxypyridazine given as infrequently as two or three times a week would be of value in the prevention of streptococcal infections in patients who have had rheumatic fever. Other instances where the drug could be employed prophylactically would perhaps include family contacts of meningococcal and *Shigella* infections.

In employing this new concept of sulfonamide drug administration offered

by sulfamethoxypyridazine, one must be extremely careful to emphasize to physician, nurse, and parent alike the fact that a *single daily dose* is to be employed rather than the conventional 6- to 8-hour dosage schedule. Even in this small series we have seen the following errors made in this regard: (1) the total daily dose was divided into 4 equal doses by an uninformed physician; (2) the dose was transcribed from q.d. to q.i.d. by an uninformed nurse; and (3) a daily dose was omitted by a forgetful parent.

Notwithstanding the necessity of learning a new and quite different dosage schedule, sulfamethoxypyridazine may well prove to be a valuable addition to the antibacterial therapeutic armamentarium of the pediatrician.

Acknowledgment

We are indebted to Charles Tappan for his technical assistance.

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CLINICAL EXPERIENCE WITH SULFAMETHOXYPYRIDAZINE*

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Sulfamethoxypyridazine (Kynex†) has been shown to have antibacterial activity approximately equivalent to that of sulfadiazine. However, it possesses certain additional characteristics that make it more desirable for clinical usage. It is well absorbed from the gastrointestinal tract, poorly acetylated, excreted very slowly, and quite soluble in urine. Frisk and Wassen[1] and Nichols *et al.*[2] have demonstrated significant blood levels for 4 days following a single 4.0-gm. dose, 15 per cent or less of the drug being acetylated. However, from 35 to 60 per cent of the amount excreted was in the conjugated form. No signs of renal or hematological toxicity have been reported, although an occasional patient experienced headache and malaise, and a few instances of skin rash have been noted.

Sulfamethoxypyridazine was used in the treatment of 16 cases of acute pyelonephritis or acute exacerbation of chronic pyelonephritis in dosages of 1.0 to 2.0 gm. initially, and maintenance doses of 1.0 gm. once a day, 1.0 gm. twice daily, or 0.5 gm. twice a day. No significant differences were observed in clinical response to these different schedules.

Treatment of Acute Pyelonephritis

Ten patients who had no history or findings suggestive of pre-existing renal disease were classified as acute pyelonephritics. The initial culture of 7 patients yielded *Escherichia coli*, 4 with coexisting *Proteus mirabilis*, paracolon bacillus, or nonhemolytic streptococci. All showed rapid clinical improvement and good laboratory response although, unfortunately, 3 of the post-treatment urine cultures were discarded. The eighth patient, admitted with congestive heart failure, developed fever, pyuria, and albuminuria, but the urine culture was reported negative. She was treated with sulfamethoxypyridazine with a rapid defervescence and clearing of urine. The ninth patient had *E. intermedium* and paracolon bacillus, and the tenth had hemolytic *Staphylococcus aureus* in urine cultures. Both latter patients responded well.

An illustrative case was that of a 28-year-old woman (P.C. 144256), admitted to the Cincinnati General Hospital in October 1956 with chills, fever, flank and back pain, and dysuria. She had had 3 episodes of post-partum endometritis during the past 5 years. There was no previous history of urinary symptoms, and a urine culture during an attack of endometritis was negative. Rather severe costovertebral angle tenderness and mild low abdominal tender-

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† Supplied by Stanton M. Hardy of the Lederle Laboratories Division, American Cyanamid Company.

TABLE 1

SULFAMETHOXYPYRIDAZINE TREATMENT OF RECURRENT PYELONEPHRITIS DUE TO *ESCHERICHIA COLI*

Date 1957	Bacteria/cc. urine	Sulfamethoxy-pyridazine (grams/day)	Sulfamethoxypyridazine blood level (mg. %)		Sulfamethoxypyridazine concentration in random urine specimens (mg. %)	
			Free	Total	Free	Total
1/31	2,500,000	0	—		—	
2/15	13,000,000	1	—		0	0
2/16	620	1			10	49
2/18	10	1			4	13.5
2/20	9	1	4.5	4.5		
3/1	18	1	4.9	6.6	7.6	37.2
3/12	0	1	3	5.1	1	7.0

ness were noted on admission, with a leukocytosis, albuminuria, and heavy pyuria. Urine culture yielded abundant *E. coli* and paracolon bacillus sensitive to Gantrisin* and Triple Sulfa*. The patient was treated with 2.0 gm. of sulfamethoxypyridazine initially, followed by 0.5 gm. twice a day. Rapid improvement in symptoms and urinalyses occurred, although pelvic tenderness persisted without cervical discharge. After 8 days of the drug and 2 negative urine cultures, tetracycline was added to treat the pelvic inflammatory disease, which responded slowly. One week after discharge the patient was asymptomatic, and a third negative urine culture was obtained.

Treatment of Chronic Pyelonephritis

Of the 6 patients with acute exacerbations of chronic pyelonephritis, 2 due to *E. coli* showed excellent clinical and laboratory responses, although one patient has not returned for follow-up. Another patient with *E. coli* pyelonephritis was reported as follows:

A 51-year-old man (W.M. 335671) was admitted with hypertensive cardiovascular disease and exacerbation of apparent chronic pyelonephritis due to *E. coli*. He responded clinically and with negative urine cultures to 3 weeks of sulfamethoxypyridazine treatment. However, 4½ months later he displayed asymptomatic gross pyuria and bacilluria due to an *E. coli* with the same antibiotic "sensitivities" as the initial organism. Again he was given 1 gm. of sulfamethoxypyridazine daily, since the colon bacillus proved sensitive to Triple Sulfa and Gantrisin. Quantitative bacterial counts on urine cultures were obtained and are shown in TABLE 1.

Only 1 of 3 patients with chronic pyelonephritis caused by *Aerobacter aerogenes* responded satisfactorily. The first was a 41-year-old woman (R.J. 334952) with a history of 4 previous attacks of pyelonephritis treated in another hospital. She was admitted after 2 weeks of dysuria and 1 day of back pain, chills, and fever. Her physical examination revealed flank tender-

* The sensitivity tests were performed by the "disc" method, employing Mueller-Hinton agar and Difco discs. Sulfamethoxypyridazine discs were not available.

ness bilaterally, and the urinalyses showed pyuria and bacilluria with *A. aerogenes*. Treatment with 0.5 gm. every 12 hr. resulted in marked clinical improvement, but the pyuria and albuminuria persisted. On the eighth day, when symptoms and high fever recurred, urine cultures yielded *E. intermedium* and *A. aerogenes*. Both organisms were resistant *in vitro* to sulfonamides, but were sensitive to chloramphenicol. The latter brought about symptomatic response, but pyuria persisted and *A. aerogenes* was still present in urine cultures.

The second failure occurred in a 68-year-old woman (M.H. 97992) admitted with an acute exacerbation of chronic pyelonephritis. The urine culture yielded *Klebsiella* type 54, sensitive *in vitro* to sulfonamides. The patient improved when given sulfamethoxypyridazine, but pyuria continued. She then developed pneumonia, with urinary incontinence necessitating an indwelling catheter. After the catheter was in place, a culture yielded *Klebsiella* type 19, resistant to sulfonamides. On another admission 3 months later her urine contained albumin, pus, and *A. aerogenes* (*Klebsiella*, type undetermined) sensitive to sulfonamides.

The successful case was that of a 20-year-old Negro woman (M.S. 29293) with a past history of post-partum pyelonephritis. She was admitted to the Outpatient Department in October 1956 with a recurrence of her infection, due to *A. aerogenes*. The organism was sensitive to Gantrisin and Triple Sulfa *in vitro*. She was treated with Gantrisin for 1 month, but symptoms recurred 2 weeks later. Repeat cultures showed *A. aerogenes* resistant to Gantrisin and Triple Sulfa. She was then given 2.0 gm. of sulfamethoxypyridazine and started on a regimen of 1.0 gm. every morning, before results of sensitivity tests were reported. She did well, with repeated negative urine cultures and urinalyses up to 4½ months. On the 11th day she developed a generalized itching and a maculopapular rash that subsided 2 days after withdrawal of the drug.

Thus, infections due to *E. coli* responded much better to sulfamethoxypyridazine than those due to *A. aerogenes*. None of the patients with acute pyelonephritis were infected with *A. aerogenes*.

Five patients with indwelling catheters were given sulfamethoxypyridazine for 9 to 37 days, either "prophylactically" or because of pyuria. *A. aerogenes* was found 3 times, and *Proteus, E. coli, and E. intermedium* once each, either alone or in combination with *A. aerogenes*. In all posttreatment cultures, the *Proteus* and *A. aerogenes* persisted. The latter made its appearance in one patient with negative pretreatment culture and in another whose *E. coli* was eradicated.

Treatment of Acute Lung Abscess

One patient with 2 large lung abscesses was successfully treated with sulfamethoxypyridazine. He was F.M. 333293, a 53-year-old Negro man who was found unconscious by the police and admitted to the Neurosurgical Service of the Cincinnati General Hospital (FIGURE 1). He was found to have 2 subdural hematomata, which were evacuated one week later. Postopera-

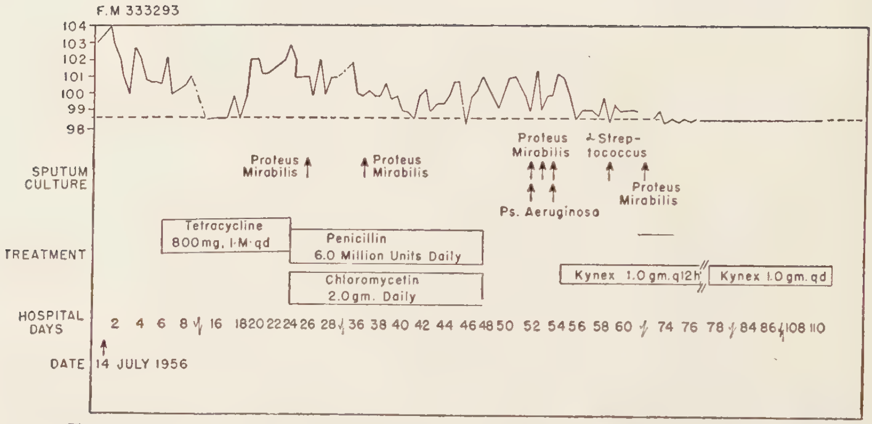

FIGURE 1. Treatment of *Proteus* lung abcess with sulfamethoxypyridazine.

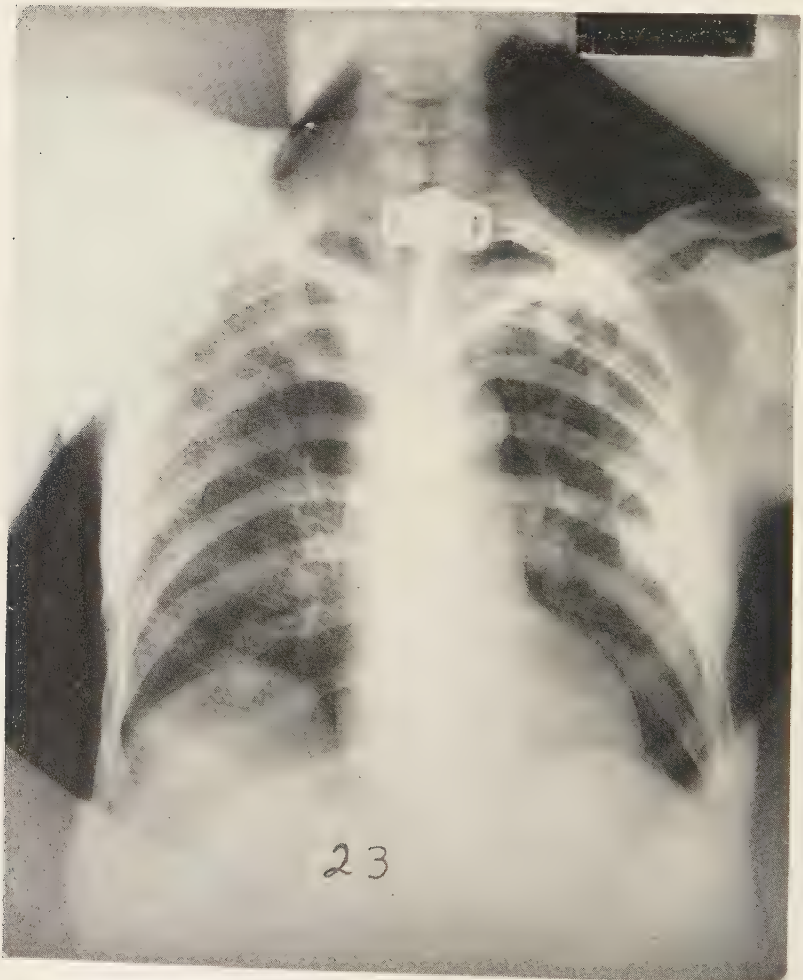

FIGURE 2

tively he was given 800 mg. of tetracycline intramuscularly every day. A tracheostomy, performed on admission, required frequent suctioning.

On the 17th day the patient began to run fever. Rales were heard in both lung fields. A chest X ray (FIGURE 2) revealed some infiltrate in the right upper lobe and 2 rib fractures on the left. Fever as high as 103° F. persisted, with a worsening of the patient's clinical state. On the 24th hospital day penicillin (6.0 million units) and chloramphenicol (2.0 gm.) daily were substituted for tetracycline. Sputum smears from the profuse tracheostomy drainage revealed large numbers of Gram-negative rods, which a culture showed to be *Proteus mirabilis*. Another chest X ray on the 28th day (FIGURE 3) showed bronchopneumonia on the right with a large abscess cavity in the right midlung field. By the 42nd day (FIGURES 4 and 5), this cavity was

FIGURE 3

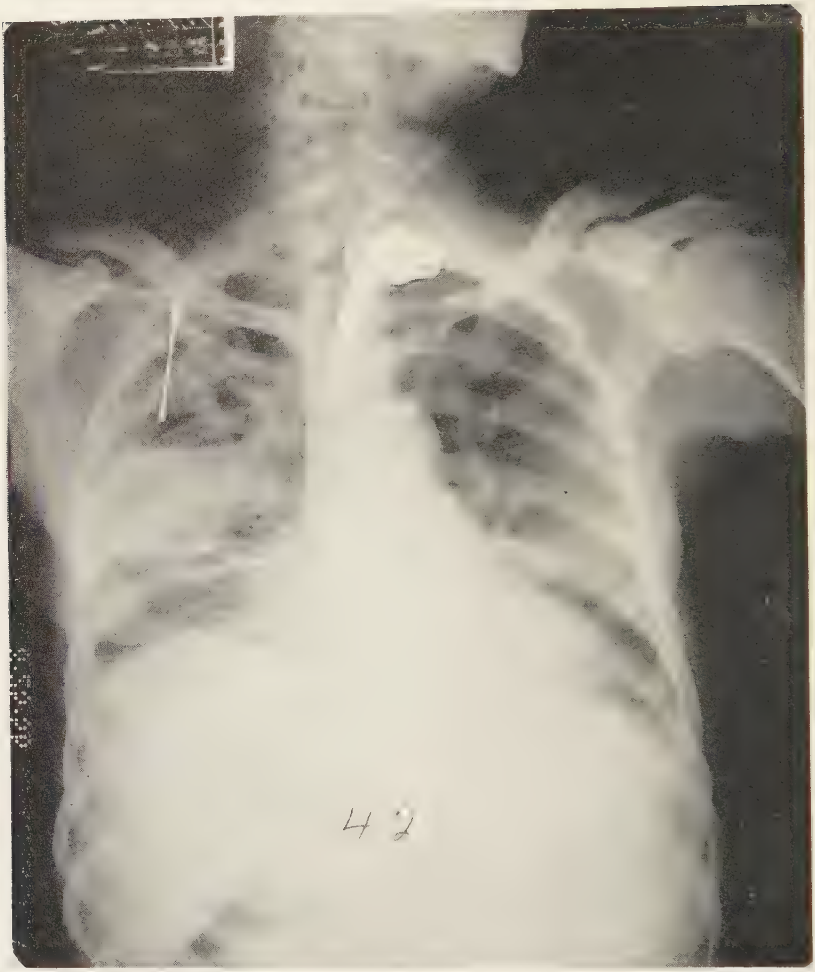

FIGURE 4

much larger, and a second cavity was seen in the right middle lobe anteriorly. Repeated cultures of the sputum yielded *Proteus mirabilis* resistant to all available antibiotics. Profuse thick white sputum and fever persisted despite penicillin, chloramphenicol, and postural drainage. On the 47th day he was transferred to the Medical Service for treatment of the lung abscesses.

All medication was discontinued for 1 week. The fever persisted at about the previous level, and the X-ray appearance of the abscesses remained unchanged (FIGURE 6). *Proteus mirabilis*, resistant to all available antibiotics, but sensitive to Triple Sulfa and Gantrisin, were repeatedly recovered from the sputum. On the 55th day, treatment with sulfamethoxypyridazine was begun on a schedule of 2.0 gm. initially and 1.0 gm. every 12 hr. A prompt

FIGURE 5

drop in temperature occurred and, 5 days later, the patient was afebrile and remained so throughout his hospital course. Three weeks after the initiation of sulfamethoxypyridazine therapy the abscess cavities were smaller (FIGURE 7), and the dosage was reduced to 1.0 gm. every morning. The profuse discharge of sputum from the tracheostomy decreased markedly during the first week and, by about 4 weeks after beginning sulfonamide therapy, it had gradually disappeared. Seven weeks after starting sulfamethoxypyridazine the chest X ray showed no residua (FIGURE 8), and drug therapy was discontinued after a total of 55 days of sulfamethoxypyridazine. The patient was observed for an additional 4 weeks in the hospital, during which time a herniorrhaphy was performed without complications. He has been followed in clinic for an

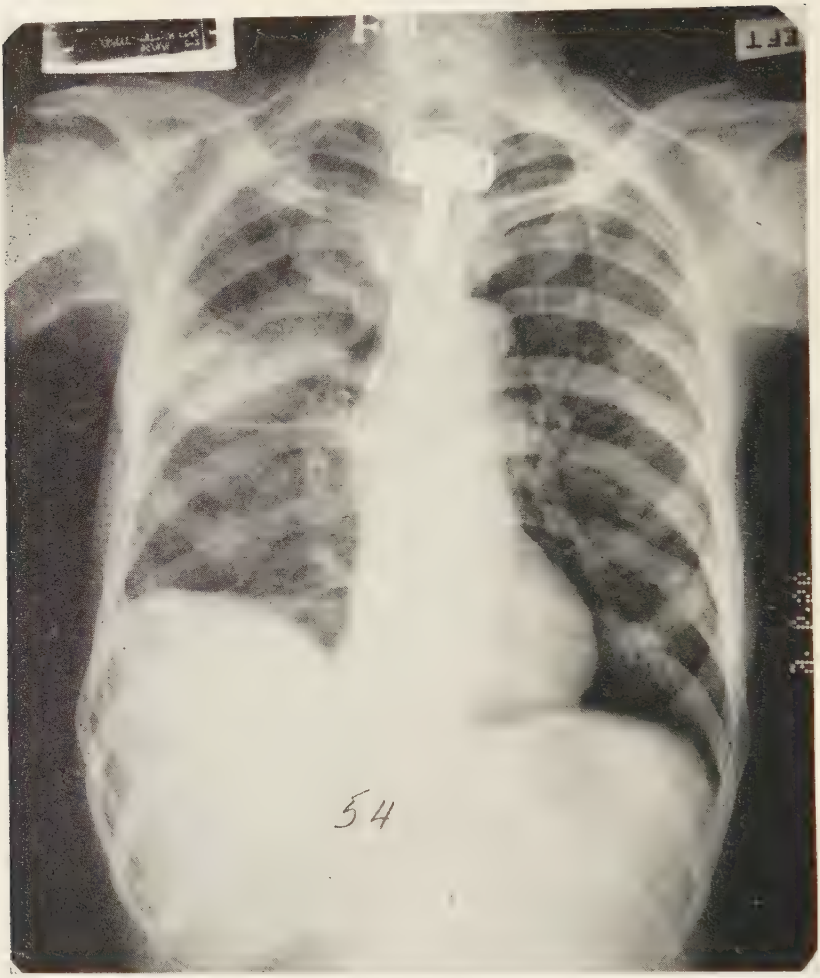

FIGURE 6

additional 3 months without noting symptoms referable to the chest. At no time were leukopenia or crystalluria noted.

Blood and Urine Levels

Sulfonamide levels were determined by the method of Bratton and Marshall.[3] Whole blood levels were measured every 24 hr. for 4 days in 4 subjects, after administration of 2.0 gm. of sulfamethoxypyridazine. The experiment was performed twice in 2 of the subjects. The average levels and ranges are given in TABLE 2. The figures in parentheses represent the upper and lower limits in each group.

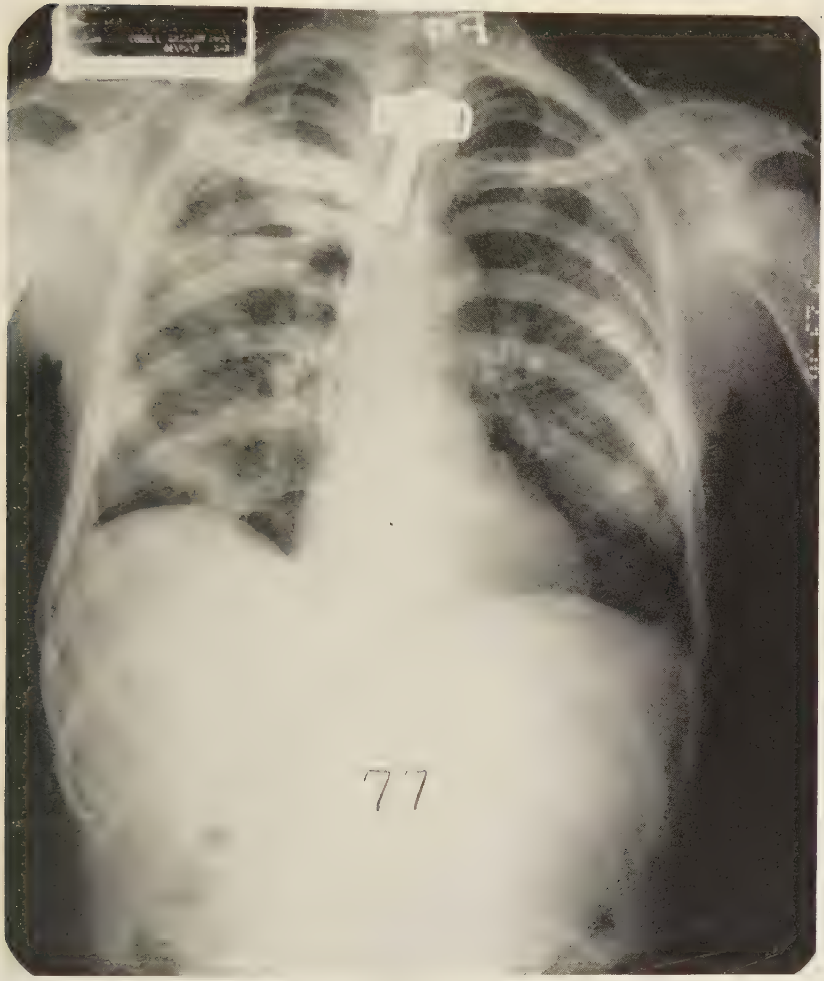

FIGURE 7

The average blood levels of free and total sulfonamide in patients given daily doses of 0.5 gm. every 12 hr., or 1.0 gm. each morning, are listed in TABLE 3.

It will be noted that the trough levels are not substantially different on the 2 schedules. The variation in blood levels from patient to patient was greater than that found from day to day in the same patient. For example, while receiving 1.0 gm. of sulfamethoxypyridazine daily, one subject had levels of free sulfonamide from 3.9 to 5.0 mg. per cent, while another subject of approximately the same weight had levels from 10.1 to 12.4 mg. per cent. The average amount of conjugated sulfonamide in the blood has been found to range from 5 to 19 per cent of the total level.

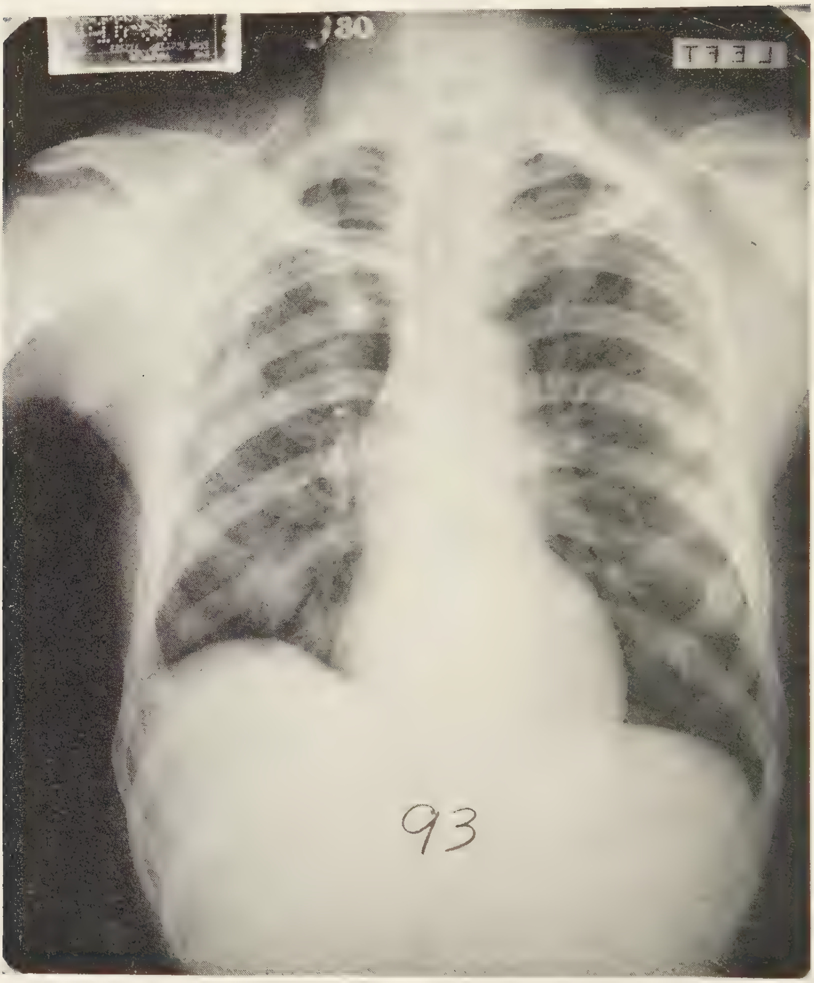

FIGURE 8

TABLE 2

	24 hr.	48 hr.	72 hr.	96 hr.
Free, mg. %	5.3 (1–9.25)	5.2 (3.9–6.5)	3.1 (1.4–4.4)	1.3 (1–2.7)
Total, mg. %	5.9 (2.4–10.0)	5.3 (2.4–6.9)	3.3 (1.6–4.8)	1.5 (1–2.5)

Similar levels for 2 patients treated with 1.0 gm. every 12 hr. are presented in TABLE 4.

Twenty-four-hour urine specimens were collected on 7 subjects receiving sulfamethoxypyridazine daily. From 29.4 to 70.4 per cent of the excreted

Table 3
Trough Blood Levels of Sulfamethoxypyridazine in Subjects Given 1 Gm. Per Day

Dose	No. of subjects	Average (mg. %)	Range
0.5 gm. q. 12 h.	9	5.15 (free)	1.3–10.1
1.0 gm. q. 24 h.	12	6.21 (free)	2.0–12.4
	8	7.71 (total)	2.0–12.2

Table 4
Trough Blood Levels in 2 Patients Given 1 Gm. of Sulfamethoxypyridazine Every 12 Hr.

Patient	No. determinations	Average level	Range
1	6	7.85 (free)	6.5–8.5
2	4	18.1 (free)	16.7–20
	4	18.5 (total)	16.6–21.2

drug was in the conjugated form. Although the collection of urine was carried out by the patients themselves, without strict supervision, it is noteworthy that, in the only 2 patients in whom 96-hour collections were obtained, 89.6 and 89.5 per cent of the drug was recovered. This high recovery rate has been mentioned by others.

Toxicity

To date we have administered sulfamethoxypyridazine to 52 subjects on various schedules (not exceeding 2.0 gm. daily) for 1 to 55 days. In many of these the clinical effectiveness of the drug could not be evaluated. We have witnessed the appearance of a generalized pruritic maculopapular eruption on the 11th day of administration in 1 patient. The rash subsided 2 days after the drug was discontinued. An occasional patient has complained of headache and malaise, but these symptoms have not been severe. In none have we detected crystalluria or leukopenia. There have been no gastrointestinal reactions.

Comment

Sulfamethoxypyridazine has the desirable properties of good gastrointestinal absorption, slow renal excretion, and sustained high blood levels with low acetylation. The blood levels we have obtained are similar to those of Foerster *et al.*[4] of the Mayo Clinic, Rochester, Minn., and not as high as those reported by others.[1] Nevertheless, there is individual variation, some patients maintaining high levels and others somewhat lower levels on the same dosage schedule. There was practically no difference between 0.5 gm. twice a day and 1.0 gm. once a day.

Despite the prolonged blood level and slow rate of excretion following a single dose, there was no accumulation in the blood when the drug was continued for as much as 55 days. Two patients whose urinary sulfamethoxypyridazine excretion was studied for 4 consecutive days displayed little varia-

tion in daily excretion. We did not notice any correlation between the blood level and the amount of the drug excreted in the patients' urine.

Our experience in the treatment of pyelonephritis reflects what the last 20 years have taught us to expect of sulfonamide therapy. In uncomplicated cases, caused by sulfonamide-sensitive bacteria, the results with sulfamethoxypyridazine were good. Where sulfonamide-resistant organisms were present, or when indwelling catheters were in place, the results were poor.

The most dramatic response to sulfamethoxypyridazine therapy was in the treatment of a patient with 2 large lung abscesses caused by *Proteus mirabilis* resistant to all available antibiotics. This patient serves as a reminder that infections by some strains of *Proteus* may still be successfully treated with the sulfonamides.

Summary

Sulfamethoxypyridazine was administered to 52 persons for periods ranging from 1 to 55 days. An occasional headache and one instance of maculopapular rash were the only demonstrable toxic effects.

Patients maintained on 1 gm. each morning following a loading dose of 2 gm. had average trough whole blood levels of 6.2 mg. per cent free and 7.2 mg. per cent total drug. The range of free drug levels varied from 2 to 12.4. Following a single oral dose of 2 gm., the following average free blood levels were observed: 24 hr., 5.3 mg. per cent; 48 hr., 5.2 mg. per cent; 72 hr., 3.1; 96 hr., 1.3.

The drug was used effectively in the treatment of 10 cases of acute pyelonephritis, most of which were caused by *E. coli*; it was much less effective against strains of *A. aerogenes*. One patient with *Proteus* lung abscesses was cured.

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CLINICAL USE OF SULFACHLOROPYRIDAZINE

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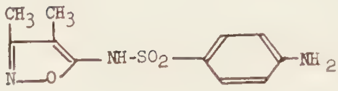
The purpose of this report is to present our experiences with a new sulfonamide, a pyridazine derivative, for the treatment of chronic genitourinary infections. The chemical configuration of this compound, 3-chloro-6-sulfanilamido-pyridazine or sulfachloropyridazine, known as Ba-10370, is presented in FIGURE 1. For comparison, the chemical structure of sulfisoxazole (Gantrisin), sulfadiazine, and sulfadimetine (Elkosin) are also shown. Although not indicated in the figure, the other available pyridazine, sulfamethoxypyridazine (Kynex), discussed by previous contributors to this monograph, differs in that a methoxy radical replaces the chloride in Ba-10370.

Selection of Patients and Procedure

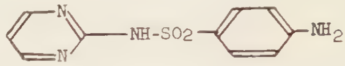
All patients were inmates of a chronic disease hospital for a variety of medical conditions. There were 26 patients, consisting of 18 females and 8 males. The age ranged between 30 and 80 years, with the majority over the age of 60. Six male patients had hypertrophied prostates, 2 patients having had a previous prostatectomy; 8 had chronic neurological conditions such as hemiplegia, paraplegia, or multiple sclerosis; 6 were bedridden because of advanced rheumatoid arthritis; and the remainder had generalized arteriosclerosis with and without hypertensive cardiovascular disease and congestive heart failure. Five of the entire group had a permanent indwelling catheter. All patients had a chronic cystitis, pyelonephritis, or both; in 6 instances, an acute exacerbation was superimposed. At least 2 of the following symptoms, dysuria, frequency, abdominal distress, or flank pain with an occasional febrile episode, were present in each case. In all cases, examination of the urine was consistent with the diagnosis of chronic genitourinary infection. Urine cultures were taken at onset of therapy under strict aseptic conditions. The selection of patients was on the basis of presence of genitourinary infection that required antibacterial therapy, and not upon the type of microorganism isolated or its sensitivity to any specific antimicrobial agent. With few exceptions, all patients had been under previous observation with other therapeutic measures. All but one patient began therapy with 0.5 gm. 3 times daily and, in 11 instances, the dose was increased to 3.0 gm. daily. The culture of the urine was repeated at peak of clinical effect and frequently thereafter. The period of observation ranged from 8 days to 3 months of continuous therapy. Patients on long-term therapy were followed for possibility of cumulative toxicity, gastrointestinal tolerance, and bacteriological resistance.

Results

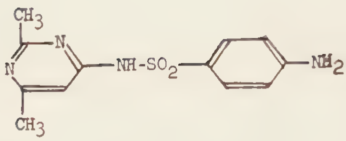
The effectiveness of Ba-10370 was based entirely upon clinical criteria[1] and is summarized in TABLE 1. A complete response indicated that all signs and



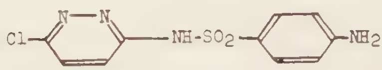
Gantrisin



Sulfadiazine



Elkosin



Ba-10370

FIGURE 1

symptoms subsided regardless of bacteriological flora. A partial but still satisfactory response indicated persistence of mild but nondistressing complaints and continuation of slight albuminuria or abnormal urinary sediment. Thus, 21 patients (84 per cent) with an initial dose of 1.5 gm. daily noted clinical improvement. Seventeen had complete control, and 4 had partial control of all signs and symptoms of genitourinary infection. Clinical effectiveness was noted, usually from 1 to 9 days, with the majority responding within the first 4 days. The higher dosage of 3 gm. daily increased the effectiveness to 92 per cent.

TABLE 1
RESPONSIVENESS OF GENITOURINARY INFECTIONS OF HOSPITALIZED PATIENTS TO Ba-10370 ADMINISTERED ORALLY REGARDLESS OF MICROORGANISMS AND SENSITIVITY

	Dosage			
	0.5 gm. t.i.d.		1.0 gm. t.i.d.	
	Number of patients	Per cent	Number of patients	Per cent
Complete	17	68.0	9	75.0
Partial	4	16.0	2	16.6
		84.0		91.6
None	4	16.0	1	8.4
	25*		12	

* One patient began therapy with 1.0 gm. t.i.d.

TABLE 2

RESPONSIVENESS OF GENITOURINARY INFECTIONS OF HOSPITALIZED PATIENTS TO Ba-10370 ADMINISTERED ORALLY ACCORDING TO SENSITIVITY OF BACTERIOLOGICAL FLORA

Sensitivity of microorganism	Number of patients	Responsiveness				Clinical control	
		Clinical and bacterio-logical	Clinical only	Clinical and partial bac-teriological	No clinical or bacterio-logical	Number of patients	Per cent
Positive	9	4	3		2	7	77.7
Mixed	4			4		4	100.0
Negative	5		5			5	100.0
Undetermined	8		7		1	7	87.5
Total	26	4	15	4	3	23	88.4

TABLE 3

RESPONSIVENESS OF GENITOURINARY INFECTIONS IN HOSPITALIZED PATIENTS TO Ba-10370 ADMINISTERED ORALLY ACCORDING TO BACTERIOLOGICAL FLORA

Microorganism*	Number of patients	Responsiveness			Clinical control	
		Clinical and bacterio-logical	Clinical only	No clinical or bacterio-logical	Number of patients	Per cent
E. coli	6	2	4		4	66.6
Enterococci	2		2		2	—
P. vulgaris	19	1	15	3	16	84.2
A. aerogenes	4	3		1	3	75.0
Ps. aeruginosa	3	1	1	1	2	66.6
H. enterococci	2	1	1		2	—
M. aureus	2		2		2	—
M. albus	1	1			1	—
A. *Streptococcus*	1			1		—
B. *Streptococcus*	4		3	1	3	75.0

* More than one microorganism might be present upon urine culture.

If the response is correlated to bacteriological flora and sensitivity of the microorganism to sulfonamide therapy, it is possible to classify the clinical effectiveness according to several categories. This is indicated in TABLE 2 by the responsiveness dependent upon the association of clinical or bacteriological effects. According to this scheme, the results of the use of Ba-10370 indicate that clinical control of the genitourinary infection occurred to the same degree regardless of the initial sensitivity of the microorganism. If the data are presented in terms of microorganisms found in the control urine culture (TABLE 3), it is again apparent that clinical control occurred regardless of microorganism. It is of interest to point out that, in 19 instances in which *Proteus vulgaris* was present, a clinical result occurred in 15 cases; in 1 other there was a combined clinical and bacteriological effect.

In 8 instances where a clinical response resulted from the 1.5-gm. daily dose without alteration in bacteriological flora, a dose increase to 3.0 gm. daily

likewise had no effect upon the microorganism. In 3 instances, however, a partial clinical response became a complete one.

None of the patients manifested any untoward reaction. Crystallization in the urine did not occur. Cumulative toxicity was not noted, although 10 patients were observed with therapy for as long as 3 months. Bacteriological resistance was not noted in those instances wherein the microorganism was eradicated from the urine. Frequent blood counts and other biochemical determinations revealed no abnormality related to drug therapy.

Discussion

This pyridazine derivative, Ba-10370, is an effective and safe agent for the treatment of chronic genitourinary infections. As we have noted previously in our laboratory,[1] there may be a discrepancy between clinical improvement for chronic genitourinary infection and bacteriological control. Since the persistence of the bacteriological flora does not appear to influence the clinical results, host factors might be an important component of the drug's clinical effectiveness.

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